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(54) Title: TRANSCOBALAMIN RECEPTOR BINDING CONJUGATES FOR NEUTRON CAPTURE THERAPY

(57) Abstract: The present invention is directed to neutron capture agents, such as a molecule comprising Boron-10 or Gadolinium-157, for the treatment, prophylaxis and/or diagnosis of a proliferative disorder via a direct or indirect attachment to a compound that binds to a transport protein for vitamin B_{12}, i.e. transcobalamin I, II or III, or intrinsic factor, (the TC- or IF-binding carrier). Subsequent initiation of neutron capture therapy will selectively destroy abnormally proliferating cells.

TRANSCOBALAMIN RECEPTOR BINDING CONJUGATES FOR NEUTRON CAPTURE THERAPY

FIELD OF THE INVENTION

The present invention includes compounds and methods for the treatment of abnormal cellular proliferation.

This application claims priority to U.S. provisional application no. 60/243,056, filed October 25, 2000.

BACKGROUND OF THE INVENTION

Cellular differentiation, growth, function and death are regulated by a complex network of mechanisms at the molecular level in a multicellular organism. In the healthy animal or human, these mechanisms allow the cell to carry out its designed function and then die at a programmed rate.

Abnormal cellular proliferation, notably hyperproliferation, can occur as a result of a wide variety of factors, including genetic mutation, infection, exposure to toxins, autoimmune disorders, and benign or malignant tumor induction.

There are a number of skin disorders associated with cellular hyperproliferation. Psoriasis, for example, is a benign disease of human skin generally characterized by plaques covered by thickened scales. The disease is caused by increased proliferation of epidermal cells of unknown cause. In normal skin the time required for a cell to move from the basal layer to the upper granular layer is about five weeks. In psoriasis, this time is only 6 to 9 days, partially due to an increase in the number of proliferating cells and an increase in the proportion of cells which are dividing (G. Grove, Int. J. Dermatol. 18:111, 1979). Approximately 2% of the population the United States have psoriasis, occurring in about 3% of Caucasian Americans, in about 1% of African Americans, and rarely in native Americans. Chronic eczema is also associated with significant hyperproliferation of the epidermis. Other diseases caused by hyperproliferation of skin cells include atopic

dermatitis, lichen planus, warts, pemphigus vulgaris, actinic keratosis, basal cell carcinoma and squamous cell carcinoma.

Other hyperproliferative cell disorders include blood vessel proliferation disorders, fibrotic disorders, autoimmune disorders, graft-versus-host rejection, tumors and cancers.

Blood vessel proliferative disorders include angiogenic and vasculogenic disorders. Proliferation of smooth muscle cells in the course of development of plaques in vascular tissue cause, for example, restenosis, retinopathies and atherosclerosis. The advanced lesions of atherosclerosis result from an excessive inflammatory-proliferative response to an insult to the endothelium and smooth muscle of the artery wall (Ross R., Nature 362:801-809 (1993). Both cell migration and cell proliferation play a role in the formation of atherosclerotic lesions.

Fibrotic disorders are often due to the abnormal formation of an extracellular matrix. Examples of fibrotic disorders include hepatic cirrhosis and mesangial proliferative cell disorders. Hepatic cirrhosis is characterized by the increase in extracellular matrix constituents resulting in the formation of a hepatic scar. Hepatic cirrhosis can cause diseases such as cirrhosis of the liver. An increased extracellular matrix resulting in a hepatic scar can also be caused by viral infection such as hepatitis. Lipocytes appear to play a major role in hepatic cirrhosis.

Mesangial disorders are brought about by abnormal proliferation of mesangial cells. Mesangial hyperproliferative cell disorders include various human renal diseases, such as glomerulonephritis, diabetic nephropathy, malignant nephrosclerosis, thrombotic microangiopathy syndromes, transplant rejection, and glomerulopathies.

Another disease with a proliferative component is rheumatoid arthritis. Rheumatoid arthritis is generally considered an autoimmune disease that is thought to be associated with activity of autoreactive T cells (See, e.g., Harris, E. D., Jr., The New England Journal of Medicine, 322: 1277-1289 (1990)), and to be caused by autoantibodies produced against collagen and IgE.

Other disorders that can include an abnormal cellular proliferative component include Behcet's syndrome, acute respiratory distress syndrome (ARDS), ischemic heart disease, post-dialysis syndrome, leukemia, acquired immune deficiency syndrome, vasculitis, lipid histiocytosis, septic shock and inflammation in general.

A tumor, also called a neoplasm, is a new growth of tissue in which the multiplication of cells is uncontrolled and progressive. A benign tumor is one that lacks the properties of invasion and metastasis and is usually surrounded by a fibrous capsule. A malignant tumor (i.e., cancer) is one that is capable of both invasion and metastasis. Malignant tumors also show a greater degree of anaplasia (i.e., loss of differentiation of cells and of their orientation to one another and to their axial framework) than benign tumors.

Approximately 1.2 million Americans are diagnosed with cancer each year, 8,000 of which are children. In addition, 500,000 Americans die from cancer each year in the United States alone. Prostate and lung cancers are the leading causes of death in men while breast and lung cancer are the leading causes of death in women. It is estimated that cancer-related costs account for about 10 percent of the total amount spent on disease treatment in the United States.CNN.Cancer.Factshttp://www.cnn.com/HEALTH/9511/conquer_cancer/facts/index.html, page 2 of 2, July 18, 1999.

Proliferative disorders are currently treated by a variety of classes of compounds including alkylating agents, antimetabolites, natural products, enzymes, biological response modifiers, miscellaneous agents, radiopharmaceuticals (for example, Yt^{90} tagged to hormones or antibodies), hormones and antagonists, such as those listed below.

Boron neutron capture therapy is based on the nuclear reaction that occurs when a stable isotope, ^{10}B, is irradiated with low energy (0.025 eV) or thermal neutrons to yield helium nuclei (α-particles) and ^{7}Li nuclei.

The therapeutic potential of this reaction was recognized by Locher over 50 years ago (Locher, G. L. et al., Am. J. Roentgenol. Radium Ther., 36, 1-13 (1936)), but it was Sweet (Javid, M. et al., J. Clin. Invest., 31, 603-610 (1952); Sweet, W. H., N. Engl. J. Med., 245, 875-878 (1951); Sweet, W. H. et al., J. Neurosurg., 9, 200-209 (1952)), who first suggested that boron neutron capture therapy (BNCT) might be useful for the treatment of brain tumors.

Shortly thereafter, a clinical trial was initiated at the Brookhaven National Laboratory in cooperation with Sweet and others at the Massachusetts General Hospital utilizing borax as the capture agent (Farr, L. E. et al., Am. J. Roentgenol., 71, 279-291 (1954); Godwin, J. T. et al., Cancer (Phila.), 8, 601-615 (1955)). The objective at that

time was to use BNCT as an adjunct to surgery for the treatment of patients with the most highly malignant and therapeutically refractory of all brain tumors, glioblastoma multiforme.

Further trials were carried out in the early 1960s, but these failed to show any evidence of therapeutic efficacy (Farr, L. E. et al., *supra*; Godwin, J. T. et al., *supra*; Asbury, A. K. et al., J. Neuropathol. Exp. Neurol., 31, 278-303 (1972)) and were associated with adverse effects in normal tissues (Asbury, A. K. et al., *supra*). Stimulated by the more encouraging clinical studies of Hatanaka et al. (Hatanaka, H. A., J. Neurol., 209, 81-94 (1975); Hatanaka, H. et al., Boron Neutron Capture Therapy for Tumors, Chap. 25, pp. 349-378. Niigata, Japan: Nishimura Co., Ltd (1986)) for the treatment of malignant gliomas and those of Mishima et al. (Mishima, Y. et al., Lancet., 2, 388-289 (1989)) for melanoma, there has been renewed national and international interest in BNCT.

The theoretical advantage of BNCT is that it is a two component or binary system, consisting of ^{10}B and thermal neutrons, which when combined together generate high linear energy transfer (LET) radiation capable of selectively destroying tumor cells without significant damage to normal tissues. In order for BNCT to succeed a critical amount of ^{10}B and a sufficient number of thermal neutrons must be delivered to individual tumor cells.

Over the past few years the Department of Energy and the NIH have renewed funding for BNCT-related research, and this has supported a growing number of investigators in many different disciplines. Advances in BNCT in the areas of compound distribution and pharmacokinetics compare favorably with other emerging modalities such as photon activation therapy, photodynamic therapy, and the use of radiolabeled antibodies for cancer treatment in which physiological targeting is used.

There are a number of nuclides that have a high propensity for absorbing low energy or thermal neutrons, and this property, referred to as the neutron capture cross-section (σ), is measured in barns (1 b = 10^{-24} cm^2). Of the various nuclides that have high neutron capture cross-sections, ^{10}B is the most attractive for the following reasons: (a) it is nonradioactive and readily available, comprising approximately 20% of naturally occurring boron; (b) the particles emitted by the capture reaction ($^{10}B(n,\alpha)^7Li$) are largely high LET; and (c) their path lengths are approximately 1 cell diameter (10-14 μm),

theoretically limiting the radiation effect to those tumor cells that have taken up a sufficient amount of ^{10}B and simultaneously sparing normal cells and (d) the extensive chemistry of boron is such that it can be incorporated into a multitude of different chemical structures.

^{7}Li and α-particles are the primary fission product of the neutron capture reaction with ^{10}B. α-Particles are relatively slow and give rise to closely spaced ionizing events that consist of tracks of sharply defined columns. They have a path length of approximately 10 μm, are high LET, and destroy a wide variety of biologically active molecules including DNA, RNA and proteins. For these reasons there is little, if any, cellular repair from α-particle-induced radiation injury.

Since the $^{10}B(n,\alpha)^{7}Li$ reaction will produce a significant biological effect only when there is a sufficient fluence of thermal neutrons and a critical amount of ^{10}B localized around, on, or within the cell, the radiation produced can be extremely localized thereby sparing normal tissue components. Thus, selectivity is simultaneously one of the advantages and disadvantages of BNCT, since it requires delivery of boron-10 to tumor cells in greater amounts than normal cells.

Ideally, boron compounds to be used for BNCT should have a high specificity for malignant cells with concomitantly low concentrations in adjacent normal tissues and blood. Since it is desirable to confine the radiation solely to these cells, an intracellular and optimally intranuclear localization of boron would be preferred.

Several boron-containing derivatives of chlorpromazine have been synthesized (Nakagawa, T. et al., Chem. -Pharm. Bull. (Tokyo), 24, 778-781 (1976); Alam, F. et al., Sthralenther. Onkol., 165, 121-125 (1989)) and are being evaluated for their *in vivo* tumor localizing properties. *p*-Boronophenylalanine is another compound that is being studied as a potential capture agent for the treatment of melanoma. The rationale for its use is the avidity of melanomas for aromatic amino acids and their subsequent incorporation into melanin (Ichihashi, M. et al., J. Invest. Dermatol., 78, 215-218 (1982); Mishima, Y. et al., Neutral Capture Therapy, 230-236, Niigata, Japan: Nishimura Co., Ltd. (1986)).

Tumor localization has been demonstrated following I.V. administration by means of whole body autoradiography (Coderre, J. A. et al., Cancer Res., 48, 6313-6316 (1988)) and in several patients with cutaneous melanoma following perilesional injection

(Mishima, Y. et al., Sthralenther. Onkol., 165, 251-254 (1989)). Stimulated by Mishima's experience, a number of other boron-containing amino acids have been synthesized that potentially could be incorporated in larger amounts into proteins of malignant cells (Hall, I. H. et al., J. Pharm. Sci., 68, 685-688 (1979).

Another approach to the selective targeting of boron to melanomas is based on the observation that thiouracil is preferentially incorporated into melanotic melanomas during melanogenesis (Whittacker, J. R., J. Biol. Chem., 246, 6217-6226 (1971)). This observation provided the impetus for the synthesis of several boron-containing thiouracils (Gabel, D., Clinical Aspects of Neutron Capture Therapy, 233-241, New York: Plenum Publishing Co. (1989)), and these currently are being evaluated in animals.

Two other classes of compounds with a propensity for localizing in malignant tumors are the porphyrins and the related phthalocyanines. The biochemical basis by which these compounds achieve elevated concentration in malignant tumors is unknown, but this observation has served as the rationale for the use of hematoporphyrin derivative in the photodynamic therapy of cancer (Dougherty, T. J. et al., Porphyrin Photosensitization, 3-13, New York: Plenum Publishing Corp. (1981)).

The high concentration of these compounds in tumors and their intracellular localization and persistence have stimulated several groups of investigators to synthesize boronated porphyrins (Kahl, S. B. et al., Neutron Capture Therapy, 61-67, Niigata, Japan: Nishimura Co., Ltd. (1986)) and phthalocyanines (Alam, F. et al., Strahlenther. Oncol., 165, 121-123 (1989)) as potential capture agents. Boronated porphyrins appear to be 3-4 times more effective per unit dose in cell culture than the monomeric or dimeric form of $Na_2B_{12}H_{11}SH$ (Laster, B. H. et al., Strahlenther. Oncol., 165, 203-205 (1989)). Although liver concentrations of these compounds are also high (Kahl, S. B. et al., *supra*) this would not limit their use as a capture agent for the treatment of brain tumors.

One final category of low molecular weight boron compounds is boron-containing purines and pyrimidines and their nucleosides. The rationale for their development is that such compounds may be selectively incorporated into rapidly proliferating tumor cells and trapped within the cell following their conversion to the corresponding nucleotide. Alternatively, these bases and their nucleosides may function as analogues of naturally occurring precursors of nucleic acids and become incorporated into nuclear DNA.

Cytoplasmic or preferably a nuclear localization of all of these boron compounds would be advantageous since the heavy particles resulting from the capture reaction would deliver a greater proportion of their energy to intranuclear targets, thereby permitting lower boron concentrations than would have been required if the compounds were located extracellularly (Gabel, D. et al., Radiat. Res., 111, 14-25 (1987); Fairchild, R. G. et al., Int. J. Radiat. Oncol. Biol. Phys., 11, 831-840 (1985)). Schinazi and Prusoff (Schinazi, R. F. et al., Tetrahedron Lett., 50, 4981-4984 (1978)) have synthesized the first boron-containing nucleoside, 5-dihydroloxyboryl-2'-deoxyuridine, an analogue of thymidine, and have shown that it was not cytotoxic to African green monkey (Vero) cells at a concentration level of 1600 μM (Laster, B. H. et al., Neutron Capture Therapy, 46-54, Niigata, Japan: Nishimura Co., Ltd. (1986)). *In vitro* neutron radiation studies of cells grown in the presence of 5-dihydroxy-2'-deoxyuridine produced a biological effect that was equivalent to a concentration of ~6 μg ^{10}B/g, which, if attainable *in vivo*, would be sufficient for BNCT.

During the 1960s and early 1970s, interests developed in the potential use of polyclonal antibodies directed against tumor-associated antigens for the delivery of drugs and radioisotopes to tumors (Pressman, D. et al., Cancer Res., 40, 3001-3007 (1957); Ghose, T. et al., Br. Med. J., 1, 90-93 (1967); Ghose, T. et al., Cancer (Phila.), 29, 1398-1400 (1972)). In 1964, Soloway suggested that antibodies might be used for the selective targeting of ^{10}B to tumors (Soloway, A.H., *supra*). Hawthorne et al. (Hawthorne, M. F. et al., J. Medicinal Chem., 15, 449-452 (1972)) reported on the incorporation of the diazonium salt from 1-(4-aminophenyl)-1,2-dicarbo-*closo*-dodecaborane into antibodies directed against bovine serum albumin and polyclonal antibodies directed against human and mouse histocompatability antigens (Hawthorne, M. F. et al., *supra*).

It was claimed from *in vitro* experiments that these immunoconjugates were capable of delivering enough boron to human and murine lymphocytes to sustain a lethal $^{10}B(n,\alpha)^{7}Li$ reaction, as evidenced by reduced viability following neutron irradiation. However, the immunoconjugates contained only 0.2% natural boron by weight, which was equal to 6 atoms of ^{10}B/molecule of antibody. In retrospect, it appears that there must have been some other explanation for the reduced cell viability that was observed. Sneath et al. (Sneath, R. L., Jr., J. Medicinal Chem., 17, 796-799 (1974)) showed that water-

solubilizing groups had to be incorporated into protein-binding polyhedral boranes if protein solubility in aqueous systems was to be maintained.

Subsequently, a group of polyhedral borane derivatives containing protein-binding functional groups were linked to IgG molecules by means of the carbodiimide reaction without evidence of precipitation (Sneath, R. L. et al., J. Medicinal Chem., 19, 1290-1294 (1976)).

One final category of macromolecular species that are possibly useful for the delivery of ^{10}B is what may be termed "encapsulating complexes," such as liposomes, microspheres and low density lipoproteins (Kahl, S. B. et al., Strahlenther. Onkol., 165, 137-139 (1989)). Theoretically, large amounts of ^{10}B could be encapsulated, and if these encapsulating complexes could be targeted to the tumor by linkage to a monoclonal antibody using existing methodology or targeting an endogenously expressed cell surface receptor, they might be powerful delivery systems. Again, there may be preferential localization in the reticuloendothelial system, and methodology would have to be developed to minimize this and maximize tumor uptake.

Two classes of compounds that are known to localize in malignant tumors are the porphyrins and the related phthalocyanines. The biochemical basis by which these compounds achieve elevated concentration in malignant tumors is unknown, but this observation has served as the rationale for the use of hematoporphyrin derivatives in the photodynamic therapy of cancer (Dougherty, T. J. *et al.*, Porphyrin Photosensitization, 3-13, New York: Plenum Publishing Corp. (1981)).

Cells undergoing rapid proliferation have been shown to increase uptake of thymidine and methionine. (See, for example, M. E. van Eijkeren *et al.*, Acta Oncologica, 31, 539 (1992); K. Kobota *et al.*, J. Nucl. Med., 32, 2118 (1991) and K. Higashi *et al.*, J. Nucl. Med., 34, 773 (1993)). Since methylcobalamin is directly involved with methionine synthesis and indirectly involved in the synthesis of thymidylate and DNA, methylcobalamin as well as Cobalt-57-cyanocobalamin have also been shown to have increased uptake in rapidly dividing tissue (for example, see, B. A. Cooper *et al.*, Nature, 191, 393 (1961); H. Flodh, Acta Radiol. Suppl., 284, 55 (1968); L. Bloomquist *et al.*, Experientia, 25, 294 (1969)). Additionally, upregulation in the number of transcobalamin II receptors has been demonstrated in several malignant cell lines during their accelerated

thymidine incorporation and DNA synthesis. See, J. Lindemans *et al.*, Exp. Cell. Res., 184, 449 (1989); T. Amagasaki *et al.*, Blood, 26, 138 (1990) and J. A. Begly *et al.*, J. Cell Physiol., 156, 43 (1993). Bacteria naturally insert Cobalt-59 into the corrin ring of vitamin B_{12}. Commercially this has been exploited by the fermentative production of Co-56, Co-57, Co-58, and Co-60 radiolabeled vitamin B_{12}. For example, see Chaiet *et al.*, Science, 111, 601 (1950). Unfortunately Cobalt-57, with a half life of 270.9 days, makes Co-57-cyanocobalamin unsuitable for clinical tumor imaging. Other metal ions (cobalt, copper and zinc) have been chemically inserted into naturally occurring descobaltocorrinoids produced by *Chromatium* and *Streptomyces olivaceous*. Attempts to chemically insert other metal ions in these cobalt free corrinoid rings have been unsuccessful. The placement of metals (cobalt, nickel, palladium, platinum, rhodium, zinc, and lithium) into a synthetic corrin ring has not presented any major difficulties. However, their instability and cost to produce makes them impractical for biological assays. Although Co–59 is has weakly paramagnetic quadrapolar nucleus in the 2^+ oxidation state, Co-59 exists in the 3^+ oxidation state within the corrin ring of vitamin B_{12} and is diamagnetic. Therefore, insertion of either a radioactive or paramagnetic metal ion other than cobalt into the corrin ring does not seem feasible at this time.

The structure of various forms of vitamin B_{12} is shown in **Figure 1**, wherein X is CN, OH, CH_3 or 5'-deoxyadenosyl, respectively. The term cobalamin is sometimes used to refer to the entire molecule except the X group. The fundamental ring system without cobalt (Co) or side chains is called *corrin* and the octadehydrocorrin is called *corrole*. **Figure 1** is adapted from The Merck Index, Merck & Co. (11th ed. 1989), wherein X is above the plane defined by the corrin ring and the nucleotide is below the plane of the ring. The corrin ring has attached seven amidoalkyl ($H_2NC(O)Alk$) substituents, at the 2, 3, 7, 8, 13, 18 and 23 positions, which can be designated a-g respectively. See D. L. Anton *et al.*, J. Amer. Chem. Soc., 102, 2215 (1980). The 2, 3, 7, 8 and 13 positions are shown in **Figure 1** as positions a-e, respectively.

For several years after the isolation of vitamin B_{12} as cyanocobalamin in 1948, it was assumed that cyanocobalamin and possibly hydroxocobalamin, its photolytic breakdown product, occurred in man. Since then it has been recognized that cyanocobalamin is an artifact of the isolation of vitamin B_{12} and that hydroxocobalamin and the two coenzyme forms, methylcobalamin and adenosylcobalamin, are the naturally occurring forms of the vitamin.

Vitamin B_{12} (adenosyl-, cyano-, hydroxo-, or methylcobalamin) must be bound by the transport protein Transcobalamin I, II, or III ("TC") to be biologically active, and by Intrinsic Factor ("IF") if administered orally. Gastrointestinal absorption of vitamin B_{12} occurs when the intrinsic factor-vitamin B_{12} complex is bound to the intrinsic factor receptor in the terminal ileum. Likewise, intravascular transport and subsequent cellular uptake of vitamin B_{12} throughout the body typically occurs through the transcobalamin transport protein (I, II or III) and the cell membrane transcobalamin receptors, respectively. After the transcobalamin vitamin B_{12} complex has been internalized in the cell, the transport protein undergoes lysozymal degradation, which releases vitamin B_{12} into the cytoplasm. All forms of vitamin B_{12} can then be interconverted into adenosyl-, hydroxo- or methylcobalamin depending upon cellular demand. See, for example, A. E. Finkler *et al.*, Arch. Biochem. Biophys., 120, 79 (1967); C. Hall *et al.*, J. Cell Physiol., 133, 187 (1987); M. E. Rappazzo *et al.*, J. Clin. Invest., 51, 1915 (1972) and R. Soda *et al.*, Blood, 65, 795 (1985).

A process for preparing ^{125}I-vitamin B_{12} derivatives is described in Niswender *et al.* (U.S. Patent No. 3,981,863). In this process, vitamin B_{12} is first subjected to mild hydrolysis to form a mixture of monocarboxylic acids, which Houts, infra, disclosed to contain mostly the (e)-isomer. The mixture is then reacted with a p-(aminoalkyl)phenol to introduce a phenol group into the B_{12} acids (via reaction with one of the free carboxylic acid groups). The mixed substituent B_{12} derivatives are then iodinated in the phenol-group substituent. This U.S. patent teaches that the mixed ^{125}I-B_{12} derivatives so made are useful in the radioimmunoassay of B_{12}, using antibodies raised against the mixture.

T. M. Houts (U.S. Patent No. 4,465,775) reported that the components of the radiolabeled mixture of Niswender *et al.* did not bind with equal affinity to IF. Houts disclosed that radioiodinated derivatives of the pure monocarboxylic (d)-isomer are useful in assays of B_{12} in which IF is used.

U.S. Patent Nos. 5,739,313; 6,004,533; 6,096,290 and PCT Publication WO 97/18231 listing Collins and Hogenkamp as inventors disclose radionuclide labeling of vitamin B_{12} through the propionamide moieties on naturally occurring vitamin B_{12}. The inventors converted the propionamide moieties at the *b-, d-, and e-* positions of the corrole ring to monocarboxylic acids, through a mild hydrolysis, and separated the carboxylic acids by column chromatography. The inventors then attached a bifunctional linking

moiety to the carboxylate function through an amide linkage, and a chelating agent to the linking moiety again through an amide linkage. The chelating moiety was then used to attach a radionuclide to the vitamin that can be used for therapeutic and/or diagnostic purposes. See also PCT Publications WO 00/62808; WO 01/28595 and WO 01/28592.

PCT Publication WO 98/08859 listing Grissom *et al* as inventors discloses conjugates containing a bioactive agent and an organocobalt complex in which the bioactive agent is covalently bound directly or indirectly, via a spacer, to the cobalt atom. The organocobalt complex can be cobalamin and the bioactive agent can be a chemotherapeutic agent. However, only one bioactive agent (i.e., chemotherapeutic agent) is attached to the organocobalt complex (i.e., cobalamin) and the attachment is solely through the cobalt atom (i.e., the 6-position of cobalamin). The bioactive agent is released from the bioconjugate by the cleavage of the weak covalent bond between the bioactive agent and the cobalt atom as a result of normal displacement by cellular nucleophiles or enzymatic action, or by application of an external signal (e.g., light, photoexcitation, ultrasound, or the presence of a magnetic field).

U.S. Patent Nos. 5,428,023; 5,589,463 and 5,807,823 to Russell-Jones *et al.* discloses a vitamin B_{12} conjugate for delivering oral hormone formulations. Russell-Jones teaches that the vitamin B_{12} conjugate must be capable of binding *in vivo* to intrinsic factor, enabling uptake and transport of the complex from the intestinal lumen of a vertebrate host to the systemic circulation of the host. The hormones are attached to the vitamin B_{12} through a hydrolyzed propionamide linkage on the vitamin. The patent states that the method is useful for orally administering hormones, bioactive peptides, therapeutic agents, antigens, and haptens, and lists as therapeutic agents neomycin, salbutamol cloridine, pyrimethamine, penicillin G, methicillin, carbenicillin, pethidine, xylazine, ketamine hydrochloride, mephanesin and iron dextran. U.S. Patent No. 5,548,064 to Russell-Jones *et al.* discloses a vitamin B_{12} conjugate for delivering erythropoietin and granulocyte-colony stimulating factor, using the same approach as the '023 patent.

U.S. Patent No. 5,449,720 to Russell-Jones et al discloses vitamin B_{12} linked through a polymer to various active agents wherein the conjugate is capable of binding to intrinsic factor for systemic delivery. In particular, the document discloses the attachment of various polymeric linkers to the propionamide positions of the vitamin B_{12} molecule,

and the attachment of various bioactive agents to the polymeric linker. Exemplary bioactive agents include hormones, bioactive peptides and polypeptides, antitumor agents, antibiotics, antipyretics, analgesics, antiinflammatories, and haemostatic agents. Exemplary polymers include carbohydrates and branched chain amino acid polymers. The linkers used in '720 are polymeric. Importantly, the linkers are described as exhibiting a mixture of molecular weights, due to the polymerization process by which they are made. See in particular, page 11, lines 25-26 wherein it is stated that the polymer used in that invention is of uncertain size and/or structure.

PCT Publication WO 99/65930 to Russell-Jones *et al.* discloses the attachment of various agents to the 5'-OH position on the vitamin B_{12} ribose ring. The publication indicates that the system can be used to attach polymers, nanoparticles, therapeutic agents, proteins and peptides to the vitamin. See also, U.S. Patent Nos. 6,262,253 "Vitamin B_{12} conjugates with GCSF, analogues thereof and pharmaceutical compositions;" 6,221,397 "Surface cross-linked particles suitable for controlled delivery;" 6,159,502 "Oral delivery systems for microparticles;" 6,150,341 "Vitamin B_{12} derivatives and methods for their preparation;" 5,869,466 "Vitamin B_{12} mediated oral delivery systems for GCSF;" and 5,548,064 "Vitamin B_{12} conjugates with EPO, analogues thereof and pharmaceutical compositions."

U.S. Patent No. 5,574,018 to Habberfield *et al.* discloses conjugates of vitamin B_{12} in which a therapeutically useful protein is attached to the primary hydroxyl site of the ribose moiety. The patent lists erythropoietin, granulocyte-colony stimulating factor and human intrinsic factor as therapeutically useful proteins, and indicates that the conjugates are particularly well adapted for oral administration.

U.S. Patent No. 5,840,880 to Morgan, Jr. *et al.* discloses vitamin B_{12} conjugates to which are linked receptor modulating agents, which affect receptor trafficking pathways that govern the cellular uptake and metabolism of vitamin B_{12}. The receptor modulating agents are linked to the vitamin at the *b-, d-, or e-* position.

Other patent filings which describe uses of Vitamin B_{12} include U.S. Patent No. 3,936,440 to Nath (Method of Labeling Complex Metal Chelates with Radioactive Metal Isotopes); U.S. Patent No. 4,209,614 to Bernstein et al., (Vitamin B_{12} Derivatives Suitable for Radiolabeling); U.S. Patent No. 4,279,859 (Simultaneous Radioassay of Folate and Vitamin B_{12}); U.S. Patent No. 4,283,342 to Yollees (Anticancer Agents and Methods of

Manufacture); U.S. Patent No. 4,301,140 to Frank et al (Radiopharmaceutical Method for Monitoring Kidneys); U.S. Patent No. 4,465,775 to Houts (Vitamin B_{12} and labeled Derivatives for Such Assay); U.S. Patent No. 5,308,606 to Wilson et al (Method of Treating and/or Diagnosing Soft Tissue Tumors); U.S. Patent No. 5,405,839 (Vitamin B_{12} Derivative, Preparation Process Thereof, and Use Thereof); U.S. Patent No. 5,449,720 to Russell-Jones et al., (Amplification of the Vitamin B_{12} Uptake System Using Polymers); U.S. Patent No. 5,589,463 to Russell Jones (Oral Delivery of Biologically Active Substances Bound to Vitamin B_{12}); U.S. Patent No. 5,608,060 to Axworthy et al (Biotinidase-Resistant Biotin-DOTA Conjugates); U.S. Patent No. 5,807,832 to Russell-Jones et al (Oral Delivery of Biologically Active Substances Bound to Vitamin B_{12}); U.S. Patent No. 5,869,465 to Morgan et al (Method of Receptor Modulation and Uses Therefor); U.S. Patent No. 5,869,466 to Russell-Jones et al (vitamin B_{12} Mediated Oral Delivery systems for GCSF).

See also Ruma Banerjee, *Chemistry and Biochemistry of B_{12}* John Wiley & Sons, Inc. (1999), and in particular Part II, Section 15 of that book, entitled "Diagnostics and Therapeutic Analogues of Cobalamin," by H. P. C. Hogenkamp, Douglas A. Collins, Charles B. Grissom, and Frederick G. West.

Despite the above findings, there remains a need for new compounds, compositions and methods to administer neutron capture agents that have improved specificity, i.e., which can localize the active agent efficiently in abnormally proliferating cells in high concentration compared to normal cells.

SUMMARY OF THE INVENTION

It has been discovered that a neutron capture agent such as a molecule comprising Boron-10, for the treatment of a proliferative disorder, is highly and effectively absorbed into a site of unwanted proliferation by direct or indirect attachment to a compound that binds to a transport protein for vitamin B_{12}, i.e. transcobalamin I, II or III, or intrinsic factor, (the TC- or IF-binding carrier) in a manner that allows binding to a transcobalamin receptor (TR). By initiating neutron capture after administration of the neutron capture agent, abnormally proliferating cells can be selectively destroyed.

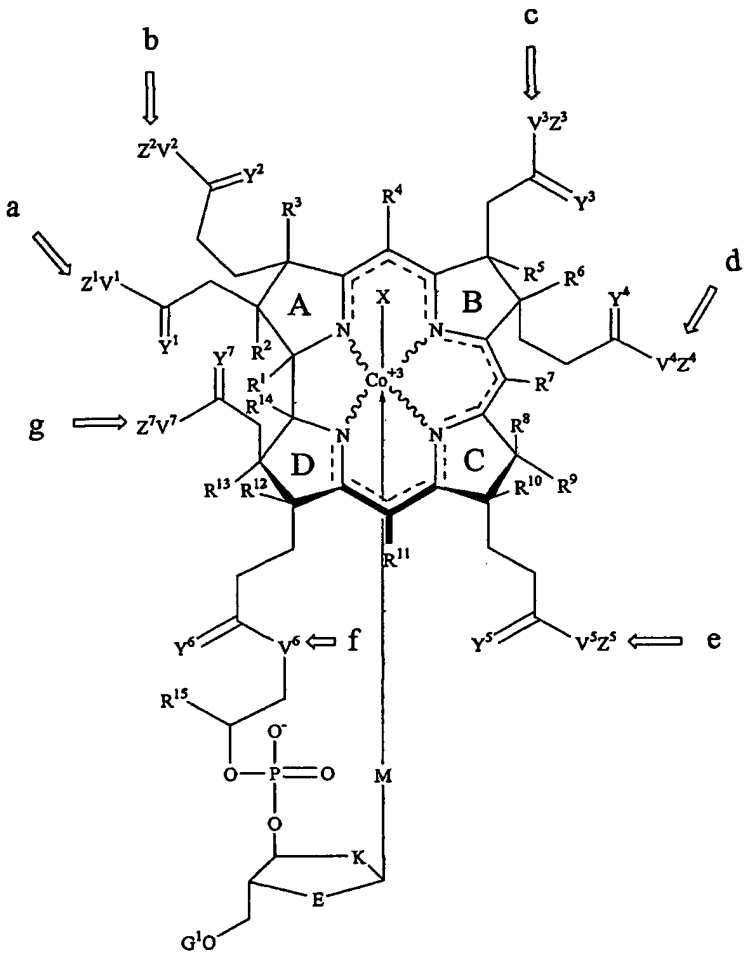
The TC-or IF-binding carrier and neutron capture agent useful to treat or image sites of proliferative disease in the body, such as cancerous tumors, can optionally be joined by means of a di- or multi-valent linking moiety. The linker used to join the TC- or IF-binding carrier and the active agent preferably has a single molecular weight, and does not exhibit a molecular weight distribution, for example as found in most polymers. The linker can range in size from small to large molecular weight, as long as there is not a distribution of weights in the linker. It is important to strictly control the uniformity of size of the conjugate for predictability of therapeutic performance.

The linkers preferably have a molecular weight below about 2000, more preferably below about 1900 or 1800, and even more preferably below about 1500 or 1000.

Thus, in one embodiment the invention provides a neutron capture conjugate having a high specificity for abnormally proliferative cells, comprising (1) a TC- or IF-binding carrier, and (2) a neutron capture agent linked directly or through a linker to the TC- or IF-binding carrier, wherein the linker has either (i) a unimodal (i.e., single) and defined molecular weight, or (ii) a molecular weight less than about 2000, and preferably, below 1900, 1800, or 1500.

In one embodiment, the TC- or IF-binding carrier is any moiety that will bind to a transcobalamin receptor and is able to be linked to a neutron capture agent. Methods for the assessment of whether a moiety binds the TC receptor are known, and include those described by Pathare, et al., (1996) *Bioconjugate Chem.* 7, 217-232; and Pathare, et al., *Bioconjugate Chem.* **8**, 161-172. An assay that assesses binding to a mixture of transcobalamin I and II receptors is found in Chaiken, et al, *Anal. Biochem.* 201, 197 (1992) An unsaturated vitamin B_{12} binding capacity (UBBC) assay to assess the *in vitro* binding of the conjugate to the transcobalamin proteins is described by D. A. Collins and H. P. C. Hogenkamp in *J. Nuclear Medicine,* 1997, *38*, 717-723. See also Fairbanks, V. F. *Mayo Clinical Proc.* 83, Vol 58, 203-204.

In another embodiment the TC- or IF-binding carrier is represented by formula (I).



(I)

or its enantiomer, diastereomer or the pharmaceutically acceptable salt or prodrug thereof, wherein:

(i) the wavy line in the chemical structure indicates either a dative or covalent bond such that there are three dative Co-N bonds and one covalent Co-N bond, wherein, in the case of the dative bond, the valance of nitrogen is completed either with a double bond with an adjacent ring carbon or with a hydrogen;

(ii) the dotted line in the chemical structure indicates either a double or single bond such that the double bond does not over-extend the valence of the element (i.e. to give pentavalent carbons) and, in the case of a single bond, the valence is

completed with hydrogen; and preferably, the bonding and stereochemistry of the compound is the same as that of vitamin B_{12} as it exists in nature;

(iii) X is hydrogen, cyano, halogen (Cl. F, Br or I), haloalkyl (including CF_3, CF_2CF_3, CH_2CF_3 and CF_2Cl), NO_2, NO_3, $PR^{15}R^{16}R^{17}$, NH_2, $NR^{15}R^{16}$, OH, OR^{15}, SR^{15}, SCN, N_3, $OC(O)R^{15}$, $C(O)_2R^{15}$, $C(O)R^{15}$, $OC(O)NR^{15}R^{16}$, $C(O)_2NR^{15}R^{16}$, $C(O)NR^{15}R^{16}$, $P(O)_2OR^{15}$, $S(O)_2OR^{15}$, a purine or pyrimidine nucleoside or nucleoside analog, including adenosyl (preferably linked through a 5'-deoxy linkage), alkyl, alkenyl, alkynyl, aryl, aralkyl, alkaryl, amino acid, peptide, protein, carbohydrate, heteroalkyl, heterocycle, heteroaryl, or alkylheteroaryl; alternatively, in one embodiment that is less preferred, X is L-T;

(iv) M is a monovalent heterocycle or heteroaromatic, which is capable of binding to the adjacent sugar ring and forming a dative bond with Co^{+3}, and is preferably a benzimidazole, a 5- and/or 6- substituted benzimidazole, such as 5,6-dimethylbenzimidazole, 5-methyl-benzimidazole, 5-hydroxybenzimidazole, 5-methoxy-benzimidazole, naphth-imidazole, 5-hydroxy-6-methyl-benzimidazole or 5-methoxy-6-methyl-benz-imidazole; or a purine or pyrimidine including but not limited to adenine, 2-methyladenine, 2-methylmercaptoadenine, e-methylsulfinyladenine, 2-methyl-sulfonyladenine and guanine; or a phenol, such as phenol or p-cresol;

(v) K is O, S, NJ^1, C(OH)H, $CR^{100}R^{101}$ or $C(R^{100})V^8Z^8$;

(vi) E is O or S;

(vii) G^1 is hydrogen, alkyl, acyl, silyl, phosphate or L-T;

(viii) Y^1, Y^2, Y^3, Y^4, Y^5, Y^6 and Y^7 independently are O, S or NJ^2;

(ix) V^1, V^2, V^3, V^4, V^5, V^6, V^7 and V^8 independently are O, S, NJ^3, $CR^{102}R^{103}$ or a direct bond;

(x) Z^1, Z^2, Z^3, Z^4, Z^5, Z^7 and Z^8 independently are R^{104} or L-T;

(xi) each L is independently a direct bond or a linker to one or more T moieties, and that does not significantly impair the ability of the TC- or IF- binding carrier to bind to a transcobalamin receptor, optionally via a transport protein;

(xii) each T independently comprises the residue of one or more molecules neutron capture agents such as a molecule comprising B-10 (in one embodiment T is for the treatment of a proliferative disorder other than cancer);

(xiii) at least one of Z^1, Z^2, Z^3, Z^4, Z^5, Z^7, Z^8, K and G^1 is L-T (in a preferred embodiment, Z^2 comprises the sole L-T in the TC- or IF- binding carrier);

(xiv) J^1, J^2 and J^3 independently are hydrogen, alkyl, alkenyl, alkynyl, alkaryl, cycloalkyl, aryl, cycloaryl, heteroalkyl, heterocycle, heteroaryl, hydroxyl, alkoxy or amine;

(xv) R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{12}, R^{13}, and R^{14} independently are hydrogen, lower alkyl, lower alkenyl, lower alkynyl, lower cycloalkyl, heteroalkyl, heterocyclic, lower alkoxy, azido, amino, lower alkylamino, halogen, thiol, SO_2, SO_3, carboxylic acid, $C_{1\text{-}6}$ carboxyl, hydroxyl, nitro, cyano, oxime or hydrazine;

(xvi) R^{13} and R^{14} optionally can form a double bond;

(xvii) R^{15}, R^{16} and R^{17} are independently hydrogen, alkyl, alkenyl, alkynyl, aryl, alkaryl or aralkyl group, heteroalkyl, heterocycle or heteroaromatic; and

(xviii) R^{100}, R^{101}, R^{102}, R^{103}, and R^{104} are independently hydrogen, alkyl, alkenyl, alkynyl, aryl, acyl, heteroaromatic, heteroaryl, heteroalkyl, hydroxyl, alkoxy, cyano, azido, halogen, nitro, SO_2, SO_3, thioalkyl, or amino.

In naturally occurring vitamin B_{12}, there is an α-D-5,6-dimethylbenzimidazolyl ribose 3'-phosphate that is bound through the phosphate to the B_{12} moiety and coordinated to the cobalt ion. In a modified vitamin B_{12} TC- or IF- binding carrier, the M-sugar component is likewise in an α-D configuration, although other configurations (i.e., α-L, β-D and β-L) are possible.

In the X position, the biologically active form of vitamin B_{12} has a 5'-deoxyadenosyl moiety. Vitamin B_{12} catalysis occurs via the detachment and reattachment of the methylene radical at the 5'-deoxy position of the adenosyl moiety. In one embodiment, the selected substituent in the X position is capable of similar catalysis.

In one particular embodiment the molecule comprising B-10 of the present invention comprises the residue of an o-, m-, or p- carborane.

In another embodiment X comprises the residue of 5'-deoxyadenosine.

In one embodiment, the TC- or IF-binding carrier comprises one or more neutron capture agents at each of one or more of the b-, d-, or e- cobalamin positions, linked directly or through a linker, and preferably through the b-position.

In another embodiment the TC- or IF-binding carrier of the present invention comprises one or more neutron capture agents at M, K or G^1.

In one embodiment, the compounds of the present invention exclude compounds (and therapeutic and imaging methods using such compounds) in which:

X is cyano, hydroxyl, methyl, adenosine or L-T,

B is the residue of 7,8-dimethylbenzimidazole,

E is O,

A is C(OH)H,

G^1 is hydrogen,

Y^1, Y^2, Y^3, Y^4, Y^5, Y^6 and Y^7 are O,

L is a direct bond or a multivalent linker derived from a dicarboxylic acid (C(O)OH-alkylene-C(O)OH), a diamine (NH_2-alkylene-NH_2), an amino-carboxylic acid (C(O)OH-alkylene-NH_2), an amino acid, a peptide or a polymer of one or amino acids,

the molecule(s) comprising B-10 is linked to the vitamin B_{12} structure through Z^2, Z^4, Z^5, X, A, or G^1,

J^1, J^2 and J^3 are all hydrogen,

all of R^1, R^2, R^4, R^5, R^8, R^9, R^{11}, R^{12} and R^{15} are methyl and all of R^3, R^6, R^7, R^{10}, R^{13} and R^{14} are hydrogen, and/or

V^1Z^1, V^3Z^3, V^6Z^6 and V^7Z^7 are amino.

The invention also provides intermediates disclosed herein that are useful in the preparation of the compounds of the present invention as well as synthetic methods useful for preparing the compounds of the invention.

The present invention also provides a method of treating a disorder characterized by abnormal cell proliferation in an animal, preferably a human, comprising administering to the animal an effective amount of a cobalamin conjugate of the present invention.

The invention also provides a method for treating and imaging a proliferative disorder in an animal, again preferably a human, comprising administering to the animal a detectable or therapeutic amount of a cobalamin conjugate of the present invention which comprises a neutron capture and imaging agent; and detecting the presence of the compound.

The invention also provides the use of a compound of the present invention for the manufacture of a medicament for treating a disorder characterized by abnormal cellular proliferation in an animal (e.g., a human).

The invention also provides a method for imaging and treating a proliferative disease (especially cancer) in a mammal (especially a human) comprising administering to the mammal a detectable amount of a cobalamin conjugate of the present invention which comprises a radionuclide; and detecting the presence of the compound.

The invention also provides the use of a compound of the present invention for the manufacture of a medicament for treating a proliferative cell disease in a mammal (e.g., a human).

The invention also provides the use of a compound of the present invention for the manufacture of a medicament for treating and imaging a proliferative disease in a mammal (e.g., a human).

In a preferred embodiment, the neutron capture agent and the TC- or IF-binding carrier, or a pharmaceutically acceptable salt or prodrug thereof, is delivered to the site of unwanted proliferation in a manner that bypasses, or at least does not rely on, the gastrointestinal route of absorption via the vitamin B_{12} intrinsic factor binding protein. Preferred modes of administration are parenteral, intraperitoneal, intravenous, intradermal, epidural, intraspinal, intrasternal, intra-articular, intra-synovial, intrathecal, intra-arterial,

intracardiac, intramuscular, intranasal, subcutaneous, intraorbital, intracapsular, topical, transdermal patch, via rectal, vaginal or urethral administration including via suppository, percutaneous, nasal spray, surgical implant, internal surgical paint, infusion pump, or via catheter. In one embodiment, the agent and carrier are administered in a slow release formulation such as a direct tissue injection or bolus, implant, microparticle, microsphere, nanoparticle or nanosphere.

In an alternative embodiment, it has been discovered that a neutron capture agent can be highly and effectively absorbed into a site of unwanted proliferation by direct or indirect attachment to a compound that binds to the intrinsic factor (IF-binding carrier), wherein the IF-binding carrier and neutron capture agent are administered parenterally, for example, using any of the methods listed above.

The TC- or IF-binding carrier and the neutron capture agent, or a pharmaceutically acceptable salt or prodrug thereof, can be administered in the course of surgical or medical treatment of the afflicted site. For example, the TC- or IF-binding carrier and active agent can be positioned directly at the site of proliferation during the course of surgery either by painting the formulation (with or without a controlled release matrix) onto the surface of the afflicted area or by depositing a bolus of material in a suitable matrix that is released into the afflicted area over time. In another embodiment, the TC- or IF-binding carrier and the active agent are administered directly into the proliferative mass via injection or catheter.

In another embodiment, the TC- or IF-binding carrier and the neutron capture agent is combined with either intrinsic factor or a transcobalamin carrier protein, or both, and administered parenterally, for example, via intravenous, intramuscular, direct injection or catheter, to the afflicted location.

It is preferred that the TC- or IF-binding carrier and the neutron capture agent be administered parenterally and not orally to increase the effectiveness of the agent. It is known that the ileal receptor for intrinsic factor-bound cobalamin is present in the gastrointestinal tract in only very small quantities, and on oral delivery of vitamin B_{12} into the alimentary system the ileal receptor can only absorb approximately two micrograms per day of vitamin B_{12} for systemic delivery. Even assuming a small amount of systemic absorption via passive transport of a large oral dose, this level of administration is insufficient for the treatment of a proliferative disorder.

In another embodiment, the TC- or IF-binding carrier and the neutron capture agent is combined with either intrinsic factor or a transcobalamin carrier protein, or both, and administered parenterally, for example, via intravenous, intramuscular, direct injection or catheter, to the afflicted location.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 depicts the structure of cobalamin wherein X is CN (cyano), OH, CH_3 or adenosyl.

Figure 2 illustrates the synthesis of a cyanocobalamin-nido-carborane conjugate.

Figure 3 illustrates the synthesis of representative compounds of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

It has been discovered that a neutron capture agent such as a molecule comprising Boron-10, for the treatment of a proliferative disorder, is highly and effectively absorbed into a site of unwanted proliferation by direct or indirect attachment to a compound that binds to a transport protein for vitamin B_{12}, i.e. transcobalamin I, II or III, or intrinsic factor, (the TC- or IF-binding carrier) in a manner that allows binding to a transcobalamin receptor (TR). Subsequent initiation of neutron capture therapy will selectively destroy abnormally proliferating cells.

In another embodiment, the TC- or IF-binding carrier and the neutron capture agent is combined with either intrinsic factor or a transcobalamin carrier protein, or both, and administered parenterally, for example, via intravenous, intramuscular, direct injection or catheter, to the afflicted location.

Therefore, the present invention includes at least the following:

(a) a TC- or IF-binding carrier of the present invention linked directly or via a linker to one or more therapeutic and/or diagnostic agent(s), including at least one neutron

capture agent, or the pharmaceutically acceptable salt or prodrug thereof, for the treatment, prophylaxis and/or diagnosis of a proliferative disorder;

(b) intermediates disclosed herein that are useful in the preparation of the compounds of the present invention as well as synthetic methods useful for preparing the compounds of the invention;

(c) a pharmaceutical composition for the treatment, prophylaxis and/or diagnosis of a proliferative disorder comprising an effective amount of a TC- or IF-binding carriers of the present invention linked directly or via a linker to one or more therapeutic and/or diagnostic agent(s), including at least one neutron capture agent, or the pharmaceutically acceptable salt or prodrug thereof, in combination with a pharmaceutically acceptable carrier of diluent;

(d) a pharmaceutical composition for the treatment, prophylaxis and/or diagnosis of a proliferative disorder comprising an effective amount of a TC- or IF-binding carrier of the present invention linked directly or via a linker to one or more therapeutic and/or diagnostic agent(s), including at least one neutron capture agent, or the pharmaceutically acceptable salt or prodrug thereof, optionally with a pharmaceutically acceptable carrier or diluent, in combination with one or more other effective therapeutic and/or diagnostic agent(s), or the pharmaceutically acceptable salt or prodrug thereof;

(e) a method for the treatment, prophylaxis and/or diagnosis of a proliferative disorder in a host, and in particular a human, comprising administering an effective amount of a TC- or IF-binding carrier of the present invention linked directly or via a linker to one or more therapeutic and/or diagnostic agent(s), including at least one neutron capture agent, or the pharmaceutically acceptable salt or prodrug thereof, optionally with a pharmaceutically acceptable carrier or diluent;

(f) a method for the treatment, prophylaxis and/or diagnosis of a proliferative disorder in a host, and in particular a human, comprising administering an effective amount of a TC- or IF-binding carrier of the present invention linked directly or via a linker to one or more therapeutic and/or diagnostic agent(s), including at least one neutron capture agent, or the pharmaceutically acceptable salt or prodrug thereof, optionally with a pharmaceutically acceptable carrier or diluent in combination or alternation

with one or more other effective therapeutic and/or diagnostic agent(s), or its pharmaceutically acceptable salt or prodrug thereof;

(g) use of an effective amount of a TC- or IF-binding carrier of the present invention linked directly or via a linker to one or more therapeutic and/or diagnostic agent(s), including at least one neutron capture agent, or the pharmaceutically acceptable salt or prodrug thereof, optionally with a pharmaceutically acceptable carrier or diluent, for the treatment, prophylaxis and/or diagnosis of a proliferative disorder in a host, and in particular a human;

(h) use of an effective amount of a TC- or IF-binding carrier of the present invention linked directly or via a linker to one or more therapeutic and/or diagnostic agent(s), including at least one neutron capture agent, or the pharmaceutically acceptable salt or prodrug thereof, optionally with a pharmaceutically acceptable carrier or diluent, in combination or alternation with one or more other effective therapeutic and/or diagnostic agent(s), or the pharmaceutically acceptable salt or prodrug thereof, for the treatment, prophylaxis and/or diagnosis of a proliferative disorder;

(i) use of an effective amount of a TC- or IF-binding carrier of the present invention linked directly or via a linker to one or more therapeutic and/or diagnostic agent(s), including at least one neutron capture agent, or the pharmaceutically acceptable salt or prodrug thereof, optionally with a pharmaceutically acceptable carrier or diluent, in the manufacture of a medicament for the treatment, prophylaxis and/or diagnosis of a proliferative disorder in a host, and in particular a human; and

(j) use of an effective amount of a TC- or IF-binding carrier of the present invention linked directly or via a linker to one or more therapeutic and/or diagnostic agent(s), including at least one neutron capture agent, or the pharmaceutically acceptable salt or prodrug thereof, optionally with a pharmaceutically acceptable carrier or diluent, in combination or alternation with one or more other effective therapeutic and/or diagnostic agent(s), or the pharmaceutically acceptable salt or prodrug thereof, in the manufacture of a medicament for the treatment, prophylaxis and/or diagnosis of a proliferative disorder.

One embodiment the invention provides a non-oral or oral pharmaceutical formulation comprising a neutron capture conjugate having a high specificity for abnormally proliferative cells, comprising (1) a transcobalamin binding carrier or an intrinsic factor binding carrier, and (2) a neutron capture agent linked directly or through a linker to the TC binding carrier or intrinsic factor binding carrier.

Another embodiment the invention provides a neutron capture conjugate having a high specificity for abnormally proliferative cells, comprising (1) a transcobalamin binding carrier or an intrinsic factor binding carrier, and (2) a neutron capture agent linked directly or through a linker to the TC binding carrier or intrinsic factor binding carrier, wherein the linker has either (i) a unimodal (i.e., single) and defined molecular weight, or (ii) a molecular weight less than about 2000, and preferably, below 1900, 1800 or 1500.

I. Definitions

The following definitions and term construction are intended, unless otherwise indicated:

Specific and preferred values listed below for radicals, substituents and ranges, are for illustration only; they do not exclude other defined values or other values within defined ranges for the radicals and substituents.

Halo is fluoro, chloro, bromo or iodo.

Alkyl, alkoxy, alkenyl, alkynyl, etc. denote both straight and branched groups; but reference to an individual radical such as "propyl" embraces only the straight chain radical, a branched chain isomer such as "isopropyl" being specifically referred to.

The term heterocycle or heterocyclic, as used herein except where noted represents a stable 5- to 7-membered monocyclic or stable 8- to 11-membered bicyclic heterocyclic ring which is either saturated or unsaturated, and which consists of carbon atoms and from one to three heteroatoms selected from the group consisting of N, O and S; and wherein the nitrogen and sulfur heteroatoms may optionally be oxidized, and the nitrogen heteroatom may optionally be quaternized, and including any bicyclic group in which any of the above-defined heterocyclic rings is fused to a benzene ring. The heterocyclic ring

may be attached at any heteroatom or carbon atom that results in the creation of a stable structure.

The term alkyl, as used herein, unless otherwise specified, refers to a saturated straight, branched, or cyclic, primary, secondary, or tertiary hydrocarbon of C_1 to C_{10}, and specifically includes methyl, ethyl, propyl, isopropyl, cyclopropyl, butyl, isobutyl, *t*-butyl, pentyl, cyclopentyl, isopentyl, neopentyl, hexyl, isohexyl, cyclohexyl, cyclohexylmethyl, 3-methylpentyl, 2,2-dimethylbutyl and 2,3-dimethylbutyl. The term includes both substituted and unsubstituted alkyl groups. Moieties with which the alkyl group can be substituted are selected from the group consisting of hydroxyl, amino, alkylamino, arylamino, alkoxy, aryloxy, nitro, cyano, sulfonic acid, sulfate, phosphonic acid, phosphate, or phosphonate, either unprotected, or protected as necessary, as known to those skilled in the art, for example, as taught in Greene, *et al.*, Protective Groups in Organic Synthesis, John Wiley and Sons, Second Edition, 1991, hereby incorporated by reference.

The term lower alkyl, as used herein, and unless otherwise specified, refers to a C_1 to C_4 saturated straight, branched, or if appropriate, a cyclic (for example, cyclopropyl) alkyl group, including both substituted and unsubstituted forms. Unless otherwise specifically stated in this application, when alkyl is a suitable moiety, lower alkyl is preferred. Similarly, when alkyl or lower alkyl is a suitable moiety, unsubstituted alkyl or lower alkyl is preferred.

The terms "alkenyl" and "alkynyl" refer to alkyl moieties wherein at least one saturated C-C bond is replaced by a double or triple bond. Thus, $(C_2$-$C_6)$alkenyl can be vinyl, allyl, 1-propenyl, 2-propenyl, 1-butenyl, 2-butenyl, 3-butenyl, 1-pentenyl, 2-pentenyl, 3-pentenyl, 4-pentenyl, 1- hexenyl, 2-hexenyl, 3-hexenyl, 4-hexenyl, or 5-hexenyl. Similarly, $(C_2$-$C_6)$alkynyl can be ethynyl, 1-propynyl, 2-propynyl, 1-butynyl, 2-butynyl, 3-butynyl, 1-pentynyl, 2-pentynyl, 3-pentynyl, 4-pentynyl, 1- hexynyl, 2-hexynyl, 3-hexynyl, 4-hexynyl, or 5-hexynyl.

The term "alkylene" refers to a saturated, straight chain, divalent alkyl radical of the formula $-(CH_2)_n-$, wherein n can be 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

As used herein, with exceptions as noted, "aryl" is intended to mean any stable monocyclic, bicyclic or tricyclic carbon ring of up to 8 members in each ring, wherein at least one ring is aromatic as defined by the Huckel 4n+2 rule. Examples of aryl ring

systems include phenyl, naphthyl, tetrahydronaphthyl and biphenyl. The aryl group can be substituted with one or more moieties selected from the group consisting of hydroxyl, amino, alkylamino, arylamino, alkoxy, aryloxy, nitro, cyano, sulfonic acid, sulfate, phosphonic acid, phosphate, or phosphonate, either unprotected, or protected as necessary, as known to those skilled in the art, for example, as taught in Greene, *et al.*, Protective Groups in Organic Synthesis, John Wiley and Sons, Second Edition, 1991.

The term purine or pyrimidine base includes, but is not limited to, adenine, N^6-alkylpurines, N^6-acylpurines (wherein acyl is C(O)(alkyl, aryl, alkylaryl, or arylalkyl), N^6-benzylpurine, N^6-halopurine, N^6-vinylpurine, N^6-acetylenic purine, N^6-acyl purine, N^6-hydroxyalkyl purine, N^6-thioalkyl purine, N^2-alkylpurines, N^2-alkyl-6-thiopurines, thymine, cytosine, 5-fluorocytosine, 5-methylcytosine, 6-azapyrimidine, including 6-azacytosine, 2- and/or 4-mercapto-pyrimidine, uracil, 5-halouracil, including 5-fluorouracil, C^5-alkylpyrimidines, C^5-benzylpyrimidines, C^5-halopyrimidines, C^5-vinylpyrimidine, C^5-acetylenic pyrimidine, C^5-acyl pyrimidine, C^5-hydroxyalkyl purine, C^5-amidopyrimidine, C^5-cyanopyrimidine, C^5-nitropyrimidine, C^5-aminopyrimidine, N^2-alkylpurines, N^2-alkyl-6-thiopurines, 5-azacytidinyl, 5-azauracilyl, triazolopyridinyl, imidazolopyridinyl, pyrrolopyrimidinyl, and pyrazolopyrimidinyl. Purine bases include, but are not limited to, guanine, adenine, hypoxanthine, 2,6-diamino-purine and 6-chloropurine. Functional oxygen and nitrogen groups on the base can be protected as necessary or desired. Suitable protecting groups are well known to those skilled in the art, and include trimethylsilyl, dimethylhexylsilyl, *t*-butyldimethylsilyl and *t*-butyldiphenylsilyl, trityl, alkyl groups, and acyl groups such as acetyl and propionyl, methanesulfonyl, and p-toluenesulfonyl.

The term heteroalkyl refers to an alkyl group that contains a heteroatom in the alkyl chain, including O, S, N, or P, and wherein the heteroatom can be attached to other substituents (including R^{15}) to complete the valence. Nonlimiting examples of heteroalkyl moieties include polyoxyalkylene, and when divalent, -(CH_2O)n- wherein n is an integer of from 0 to 20

The term acyl refers to a carboxylic acid ester in which the non-carbonyl moiety of the ester group is selected from straight, branched, or cyclic alkyl or lower alkyl, alkoxyalkyl including methoxymethyl, aralkyl including benzyl, aryloxyalkyl such as phenoxymethyl, aryl including phenyl optionally substituted with halogen, C_1 to C_4 alkyl

or C_1 to C_4 alkoxy, sulfonate esters such as alkyl or aralkyl sulphonyl including methanesulfonyl, the mono, di or triphosphate ester, trityl or monomethoxytrityl, substituted benzyl, trialkylsilyl (e.g. dimethyl-t-butylsilyl) or diphenylmethylsilyl. Aryl groups in the esters optimally comprise a phenyl group. The term "lower acyl" refers to an acyl group in which the non-carbonyl moiety is lower alkyl.

The term heteroaryl or heteroaromatic, as used herein, refers to an aromatic moiety that includes at least one sulfur, oxygen, nitrogen or phosphorus in the aromatic ring. The term heterocyclic refers to a nonaromatic cyclic group wherein there is at least one heteroatom, such as oxygen, sulfur, nitrogen or phosphorus in the ring. Nonlimiting examples of heteroaryl and heterocyclic groups include furyl, furanyl, pyridyl, pyrimidyl, thienyl, isothiazolyl, imidazolyl, tetrazolyl, pyrazinyl, benzofuranyl, benzothiophenyl, quinolyl, isoquinolyl, benzothienyl, isobenzofuryl, pyrazolyl, indolyl, isoindolyl, benzimidazolyl, purinyl, carbazolyl, oxazolyl, thiazolyl, isothiazolyl, 1,2,4-thiadiazolyl, isooxazolyl, pyrrolyl, quinazolinyl, cinnolinyl, phthalazinyl, xanthinyl, hypoxanthinyl, thiophene, furan, pyrrole, isopyrrole, pyrazole, imidazole, 1,2,3-triazole, 1,2,4-triazole, oxazole, isoxazole, thiazole, isothiazole, pyrimidine or pyridazine, and pteridinyl, aziridines, thiazole, isothiazole, 1,2,3-oxadiazole, thiazine, pyridine, pyrazine, piperazine, pyrrolidine, oxaziranes, phenazine, phenothiazine, morpholinyl, pyrazolyl, pyridazinyl, pyrazinyl, quinoxalinyl, xanthinyl, hypoxanthinyl, pteridinyl, 5-azacytidinyl, 5-azauracilyl, triazolopyridinyl, imidazolopyridinyl, pyrrolopyrimidinyl, pyrazolopyrimidinyl, adenine, N6-alkylpurines, N6-benzylpurine, N6-halopurine, N6-vinypurine, N6-acetylenic purine, N6-acyl purine, N6-hydroxyalkyl purine, N6-thioalkyl purine, thymine, cytosine, 6-azapyrimidine, 2-mercaptopyrmidine, uracil, N5-alkyl-pyrimidines, 'N5-benzylpyrimidines, N5-halopyrimidines, N5-vinyl-pyrimidine, N5-acetylenic pyrimidine, N5-acyl pyrimidine, N5-hydroxyalkyl purine, and N6-thioalkyl purine, and isoxazolyl. The heteroaromatic and heterocyclic moieties can be optionally substituted as described above for aryl, including substituted with one or more substituent selected from halogen, haloalkyl, alkyl, alkoxy, hydroxy, carboxyl derivatives, amido, amino, alkylamino, dialkylamino. The heteroaromatic can be partially or totally hydrogenated as desired. As a nonlimiting example, dihydropyridine can be used in place of pyridine. Functional oxygen and nitrogen groups on the heteroaryl group can be protected as necessary or desired. Suitable protecting groups are well known to those skilled in the art, and include trimethylsilyl, dimethylhexylsilyl, *t*-butyldi-methylsilyl, and

t-butyldiphenylsilyl, trityl or substituted trityl, alkyl groups, acyl groups such as acetyl and propionyl, methanesulfonyl, and p-toluenesulfonyl.

The term aralkyl, as used herein, and unless otherwise specified, refers to an aryl group as defined above linked to the molecule through an alkyl group as defined above. The term alkaryl, as used herein, and unless otherwise specified, refers to an alkyl group as defined above linked to the molecule through an aryl group as defined above.

The term alkoxy, as used herein, and unless otherwise specified, refers to a moiety of the structure -O-alkyl, wherein alkyl is as defined above.

The term amino, as used herein, refers to a moiety represented by the structure -NR_2, and includes primary amines, and secondary, and tertiary amines substituted by alkyl (i.e. alkylamino). Thus, R_2 may represent two hydrogens, two alkyl moieties, or one hydrogen and one alkyl moiety.

The term amido, as used herein, refers to a moiety represented by the structure -$C(O)NR_2$, wherein R_2 is as defined for amino.

As used herein, an "amino acid" is a natural amino acid residue (e.g. Ala, Arg, Asn, Asp, Cys, Glu, Gln, Gly, His, Hyl, Hyp, Ile, Leu Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, and Val) in D or L form, or an unnatural amino acid (e.g. phosphoserine; phosphothreonine; phosphotyrosine; gamma-carboxyglutamate; hippuric acid; octahydroindole-2-carboxylic acid; statine; 1,2,3,4,-tetrahydroisoquinoline-3-carboxylic acid; penicillamine; ornithine; citrulline; α-methyl-alanine; para-benzoylphenylalanine; phenylglycine; propargyl-glycine; sarcosine; and tert-butylglycine) residue having one or more open valences. Other unnatural amino acids include those represented by the formula NH_2 $(CH_2)_y$ COOH, wherein y=2-20, and preferably 2-12, and include the aminoalkanoic acids such as ε-amino caproic acid (H_2N-$(CH_2)_5$-COOH).

The term also comprises natural and unnatural amino acids bearing amino protecting groups such as acetyl, acyl, trifluoroacetyl, and benzyloxycarbonyl), as well as natural and unnatural amino acids protected at carboxy with protecting groups such as a C_1-C_6 alkyl, phenyl or benzyl ester and amide. Other suitable amino and carboxy protecting groups are known to those skilled in the art. See for example, T. W. Greene, Protecting Groups in Organic Synthesis; Wiley: New York, 1981; D. Voet, Biochemistry, Wiley: New York, 1990; L. Stryer, Biochemistry, (3rd Ed), W. H. Freeman and Co.: New

York, 1975; J. March, Advanced Organic Chemistry, Reactions, Mechanisms and Structure, (2nd Ed.), McGraw Hill: New York, 1977; F. Carey and R. Sundberg, Advanced Organic Chemistry, Part B: Reactions and Synthesis, (2nd Ed.), Plenum: New York, 1977; and references cited therein.

As used herein, a "peptide" is a sequence of 2 to 25 amino acids (e.g. as defined hereinabove) or peptidic residues having one or more open valences. The sequence may be linear or cyclic. For example, a cyclic peptide can be prepared or may result from the formation of disulfide bridges between two cysteine residues in a sequence.

As used herein, a "molecule comprising B-10" can be any compound that contains at least one B-10 atom. The nature of the molecule that includes B-10 is not critical. The compound, however, must be nontoxic and must be able to enter the tumor cell or locate near the tumor cell when the molecule comprising B-10 is attached.

As used herein, a "residue of a molecule comprising B-10" is a radical of a molecule comprising B-10 having one or more open valences. Any synthetically feasible atom or atoms of the molecule comprising B-10 may be removed to provide the open valence, provided bioactivity is substantially retained. Based on the linkage that is desired, one skilled in the art can select suitably functionalized starting materials that can be derived from a molecule comprising B-10 using procedures that are known in the art.

As used herein, a "residue of o-carborane," a "residue of m-carborane," or a "residue of p-carborane" is a radical of o-carborane, m-carborane or p-carborane, respectively, having one or more open valences. Any synthetically feasible atom or atoms of o-carborane, m-carborane or p-carborane may be removed to provide the open valence, provided bioactivity is substantially retained. Based on the linkage that is desired, one skilled in the art can select suitably functionalized starting materials that can be derived from o-carborane, m-carborane or p-carborane using procedures that are known in the art.

In general, the term "residue" is used throughout the specification to describe any pharmaceutically acceptable form of a neutron capture agent, which upon administration to a patient, does not inhibit the action of the active agent. As a non-limiting example, a pharmaceutically acceptable residue of an active agent is one that is modified to facilitate binding to the TC- or IF-binding agent, covalently, ionically or through a chelating agent, such that the modification does not inhibit the biological action of the active agent, in that

it does not inhibit the drugs ability to modulate abnormal cellular proliferation. In a preferred embodiment, the residue refers to the active agent with an open valence state such that covalent bonding to the compound is possible. This open valence state can be achieved by any means known in the art, including the methodology described herein. In a preferred embodiment, the open valence state is achieved through the removal of an atom, such as hydrogen, to activate a functional group.

As used herein, the term "substantially free of" or "substantially in the absence of" refers to a composition that includes at least 85 or 90% by weight, preferably 95% to 98 % by weight, and even more preferably 99% to 100% by weight, of the designated enantiomer of that TC- or IF-binding agent. In a preferred embodiment, in the methods and compounds of this invention, the compounds are substantially free their enantiomers.

Similarly, the term "isolated" refers to a composition that includes at least 85 or 90% by weight, preferably 95% to 98 % by weight, and even more preferably 99% to 100% by weight, of the TC- or IF-binding agent, the remainder comprising other chemical species, including diastereomers or enantiomers.

The term "independently" is used herein to indicate that the variable that is independently applied varies independently from application to application. Thus, in a compound such as R"XYR", wherein R" is "independently carbon or nitrogen," both R" can be carbon, both R" can be nitrogen, or one R" can be carbon and the other R" nitrogen.

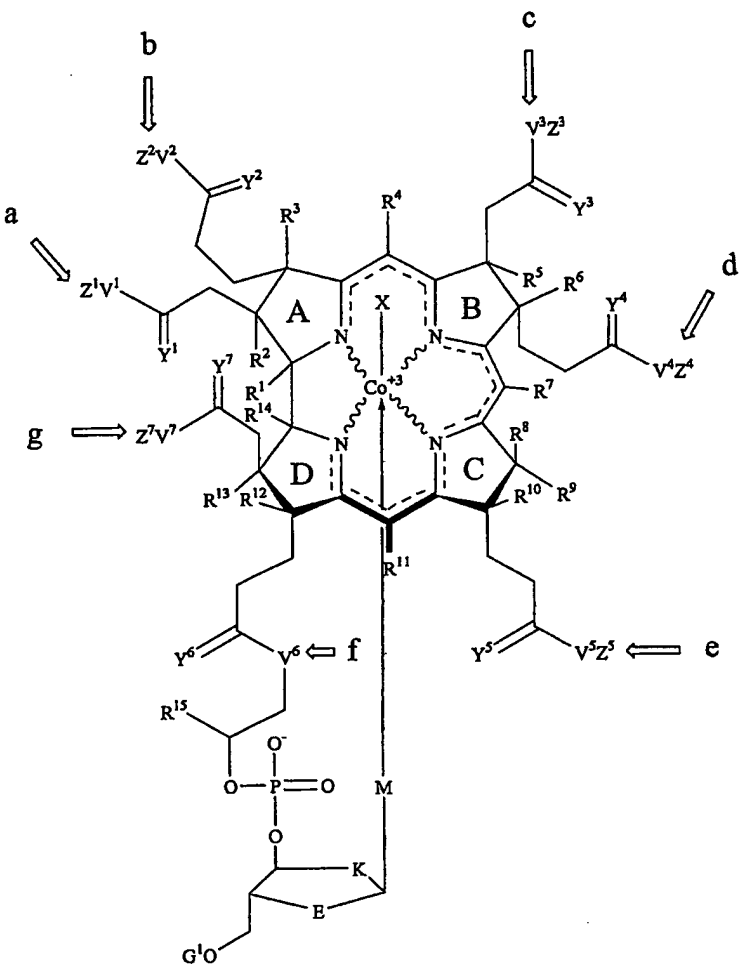
The term "host," as used herein, refers to a multicellular organism in which proliferative disorders can occur, including animals, and preferably a human. Alternatively, the host is any abnormally proliferating cell, whose replication or function can be altered by the compounds of the present invention. The term host specifically refers to any cell line that abnormally proliferates, either from natural or unnatural causes (for example, from genetic mutation or genetic engineering, respectively), and animals, in particular, primates (including chimpanzees) and humans. In most animal applications of the present invention, the host is a human patient. Veterinary applications, in certain indications, however, are clearly anticipated by the present invention (such as bovine viral diarrhea virus in cattle, hog cholera virus in pigs, and border disease virus in sheep).

II. TC-or IF-Binding Carrier Comprising B-10

In one embodiment, the TC- or IF-binding carrier is any ligand that will bind effectively to a Vitamin B_{12} transport protein (i.e. transcobalamin I, II or III or intrinsic factor) and which when appropriately linked to a neutron capture agent and bound to a transport protein, will fit into a transcobalamin receptor. Suitable carriers may be ascertained using any one of several means known in the art, including competitive binding assays with the receptor modulating agent competing with native vitamin B_{12}. Methods for the assessment of whether a moiety binds the TC receptor are known, and include those described by Pathare, et al., *Bioconjugate Chem.* **1996**, *7*, 217-232; and Pathare, et al., *Bioconjugate Chem.* **8**, 161-172. An assay that assesses binding to a mixture of transcobalamin I and II receptors is found in Chaiken, et al, *Anal. Biochem.* **1992**, *201*, 197. An unsaturated vitamin B_{12} binding capacity (UBBC) assay to assess the *in vitro* binding of the conjugate to the transcobalamin proteins is described by D. A. Collins and H. P. C. Hogenkamp in *J. Nuclear Medicine,* **1997**, *38*, 717-723. See also Fairbanks, V. F. *Mayo Clinical Proc.* 83, Vol 58, 203-204. The ligand preferably displays a binding affinity of at least 50% of the binding affinity displayed by vitamin B_{12}, more preferably at least 75% and even more preferably at least 90%.

The neutron capture agent is preferably bound directly or indirectly through an amide residue at the b-position, as illustrated in **Figure 1**.

In another embodiment the TC- or IF-binding carrier is represented by formula (I).



(I)

or its enantiomer, diastereomer or the pharmaceutically acceptable salt or prodrug thereof,

wherein:

(i) the wavy line in the chemical structure indicates either a dative or covalent bond such that there are three dative Co-N bonds and one covalent Co-N bond, wherein, in the case of the dative bond, the valance of nitrogen is completed either with a double bond with an adjacent ring carbon or with a hydrogen;

(ii) the dotted line in the chemical structure indicates either a double or single bond such that the double bond does not over-extend the valence of the element (i.e. to give pentavalent carbons) and, in the case of a single bond, the valence is

completed with hydrogen; and preferably, the bonding and stereochemistry of the compound is the same as that of vitamin B_{12} as it exists in nature;

(iii) X is hydrogen, cyano, halogen (Cl. F, Br or I), haloalkyl (including CF_3, CF_2CF_3, CH_2CF_3 and CF_2Cl), NO_2, NO_3, $PR^{15}R^{16}R^{17}$, NH_2, $NR^{15}R^{16}$, OH, OR^{15}, SR^{15}, SCN, N_3, $OC(O)R^{15}$, $C(O)_2R^{15}$, $C(O)R^{15}$, $OC(O)NR^{15}R^{16}$, $C(O)_2NR^{15}R^{16}$, $C(O)NR^{15}R^{16}$, $P(O)_2OR^{15}$, $S(O)_2OR^{15}$, a purine or pyrimidine nucleoside or nucleoside analog, including adenosyl (preferably linked through a 5'-deoxy linkage), alkyl, alkenyl, alkynyl, aryl, aralkyl, alkaryl, amino acid, peptide, protein, carbohydrate, heteroalkyl, heterocycle, heteroaryl, or alkylheteroaryl; alternatively, in one embodiment that is less preferred, X is L-T;

(iv) M is a monovalent heterocycle or heteroaromatic, which is capable of binding to the adjacent sugar ring and forming a dative bond with Co^{+3}, and is preferably a benzimidazole, a 5- and/or 6- substituted benzimidazole, such as 5,6-dimethylbenzimidazole, 5-methyl-benzimidazole, 5-hydroxybenzimidazole, 5-methoxy-benzimidazole, naphth-imidazole, 5-hydroxy-6-methyl-benzimidazole or 5-methoxy-6-methyl-benz-imidazole; or a purine or pyrimidine including but not limited to adenine, 2-methyladenine, 2-methylmercaptoadenine, e-methylsulfinyladenine, 2-methyl-sulfonyladenine and guanine; or a phenol, such as phenol or p-cresol;

(v) K is O, S, NJ^1, C(OH)H, $CR^{100}R^{101}$ or $C(R^{100})V^8Z^8$;

(vi) E is O or S;

(vii) G^1 is hydrogen, alkyl, acyl, silyl, phosphate or L-T;

(viii) Y^1, Y^2, Y^3, Y^4, Y^5, Y^6 and Y^7 independently are O, S or NJ^2;

(ix) V^1, V^2, V^3, V^4, V^5, V^6, V^7 and V^8 independently are O, S, NJ^3, $CR^{102}R^{103}$ or a direct bond;

(x) Z^1, Z^2, Z^3, Z^4, Z^5, Z^7 and Z^8 independently are R^{104} or L-T;

(xi) each L is independently a direct bond or a linker to one or more T moieties, and that does not significantly impair the ability of the TC- or IF- binding carrier to bind to a transcobalamin receptor, optionally via a transport protein;

(xii) each T independently comprises the residue of one or more molecules neutron capture agents such as a molecule comprising B-10 (in one embodiment T is for the treatment of a proliferative disorder other than cancer);

(xiii) at least one of Z^1, Z^2, Z^3, Z^4, Z^5, Z^7, Z^8, K and G^1 is L-T (in a preferred embodiment, Z^2 comprises the sole L-T in the TC- or IF- binding carrier);

(xiv) J^1, J^2 and J^3 independently are hydrogen, alkyl, alkenyl, alkynyl, alkaryl, cycloalkyl, aryl, cycloaryl, heteroalkyl, heterocycle, heteroaryl, hydroxyl, alkoxy or amine;

(xv) R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{12}, R^{13}, and R^{14} independently are hydrogen, lower alkyl, lower alkenyl, lower alkynyl, lower cycloalkyl, heteroalkyl, heterocyclic, lower alkoxy, azido, amino, lower alkylamino, halogen, thiol, SO_2, SO_3, carboxylic acid, C_{1-6} carboxyl, hydroxyl, nitro, cyano, oxime or hydrazine;

(xvi) R^{13} and R^{14} optionally can form a double bond;

(xvii) R^{15}, R^{16} and R^{17} are independently hydrogen, alkyl, alkenyl, alkynyl, aryl, alkaryl or aralkyl group, heteroalkyl, heterocycle or heteroaromatic; and

(xviii) R^{100}, R^{101}, R^{102}, R^{103}, and R^{104} are independently hydrogen, alkyl, alkenyl, alkynyl, aryl, acyl, heteroaromatic, heteroaryl, heteroalkyl, hydroxyl, alkoxy, cyano, azido, halogen, nitro, SO_2, SO_3, thioalkyl, or amino.

In naturally occurring vitamin B_{12}, there is an α-D-5,6-dimethylbenzimidazolyl ribose 3'-phosphate that is bound through the phosphate to the B_{12} moiety and coordinated to the cobalt ion. In a modified vitamin B_{12} TC- or IF- binding carrier, the M-sugar component is likewise in an α-D configuration, although other configurations (i.e., α-L, β-D and β-L) are possible.

In the X position, the biologically active form of vitamin B_{12} has a 5'-deoxyadenosyl moiety. Vitamin B_{12} catalysis occurs via the detachment and reattachment of the methylene radical at the 5'-deoxy position of the vitamin.

L is preferably a direct bond, in which the molecule comprising B-10 is directly linked to the TC carrier. However, as discussed in further detail below, the molecule comprising B-10 can also be linked through one of many suitable linkers.

Any neutron capture agent capable of absorbing low energy or thermal neutrons is suitable for practicing the invention. A preferred neutron capture agent is B-10. A variety of molecules comprising B-10 known in the art are useful in the present invention. The molecules vary considerably in structure but are suitable to practice the present invention. Acceptable species include boron containing amino acids, carbohydrates, and nucleosides, as well as carboranes. A variety of molecules comprising B-10 are commercially available from Boron Biologicals, Inc., Raleigh, North Carolina and RysCor Science, Inc., Raleigh, North Carolina.

Specifically, at least one molecule comprising B-10 can be o-carborane, m-carborane or p-carborane. More specifically, at least one molecule comprising B-10 is o-carborane. o-Carborane [1,2-dicarbadodecaborane(12)]; m-carborane [1,7-dicarbadodecaborane(12)]; and p-carborane [1,12-dicarbadodecaborane(12)] are commercially available from Aldrich, Milwaukee, WI.

Because vitamin B_{12} is preferentially taken up by abnormally proliferating cells, the TC- or IF-binding carrier/active agent of the present invention provides a delivery system capable of targeting abnormally proliferative cells, and selectively destroying a greater proportion of such cells in relation to healthy cells. A wide range of analogs and derivatives are capable of attaining these properties, as reflected by the above referenced chemical structure and variables.

The TC- or IF-binding carrier can be modified in any manner that does not interfere with its fundamental ability to bind a transcobalamin transport protein, and thereafter bind the TC receptor. In one embodiment, however, each variable on the vitamin B_{12} structure independently either (i) retains its natural vitamin B_{12} structure, (ii) imparts imaging and/or anti-proliferative properties to the cobalamin conjugate, (iii) renders the cobalamin conjugate more water soluble, or more stable, (iv) increases the bioavailability of the carrier; (v) increases or at least does not decrease the binding affinity of the carrier for the TC-binding or IF-binding protein over vitamin B_{12}; or (vi) imparts another characteristic that is desired for pharmaceutical or diagnostic performance.

The neutron capture agent can be linked to the TC-binding or IF-binding moiety through a number of positions, including any of the V-Z moieties, the X moiety, the M moiety, the K moiety and/or the G^1 moiety, though as mentioned above at least one of Z^1, Z^2, Z^3, Z^4, Z^5, Z^7, Z^8, M, K and G^1 moieties comprises a neutron capture agent. In one embodiment a neutron capture agent is linked to the TC- or IF-binding carrier through Z^2, Z^4, and/or Z^5 (i.e. one or more of Z^2, Z^4, and Z^5 is L-T, and T is a neutron capture agent). In a more particular embodiment a neutron capture agent is linked to the TC- or IF-binding carrier through the Z^2 moiety (i.e. Z^2 is L-T, and T is a neutron capture agent). In each of the foregoing embodiments, the Z moiety or moieties not containing a neutron capture agent preferably retain its natural vitamin B_{12} configuration, in which VZ is NH_2. Alternatively, the Z moieties not containing a neutron capture agent may comprise a secondary or tertiary amino analog of NH_2 substituted by one or two of J^1.

In any Z^1, Z^2, Z^3, Z^4, Z^5, Z^6, Z^7, Z^8, X, M, K, or G^1 moieties through which a neutron capture agent is linked, it will be understood that such moiety may comprise more than one neutron capture agent, or a combination of neutron capture agents, i.e., each T can independently comprise the residue of one or more neutron capture agents bound to L through one or more chelating moieties. More specifically, in a series of embodiments, each T can comprise 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 neutron capture agents bound through one or more chelating moieties.

R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{12}, and R^{13} independently represent moieties that do not interfere with binding between the compound and the transcobalamin transport protein or receptor. Vitamin B_{12} can be modified through these moieties to modulate physical properties of the molecule, such as water solubility, stability or λ_{max}. Preferred groups for enhancing water solubility include heteroalkyl, amino, C_{1-6} alkylamino, C_{1-6} alcohol, C_{1-6} carboxylic acid and SO_3^-.

In another embodiment, one, some or all of R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{12}, and R^{13} independently assume their natural roles in vitamin B_{12}. Thus, one, some, or all of R^1, R^2, R^4, R^5, R^8, R^9, R^{11}, R^{12} and R^{15} are independently methyl in one embodiment, and one, some, or all of R^3, R^6, R^7, R^{10}, R^{13} and R^{14} are independently hydrogen.

In another embodiment, one, some, or all of Y^1, Y^2, Y^3, Y^4, Y^5, Y^6 and Y^7 assume their natural roles in vitamin B_{12}, and are O. Similarly, in another embodiment V^6

assumes its natural role in vitamin B_{12}, and is NH, or a primary amine analog thereof substituted by J^1.

In still another embodiment, position X assumes its natural role in vitamin B_{12}, *i.e.* as cyano, hydroxyl, methyl or 5'-deoxyadenosyl, most preferably 5'-deoxyadenosyl.

In another embodiment M is the radical of a purine or pyrimidine base. In another embodiment M is the radical of adenosine, guanine, cytosine, uridine or thymine. In still another embodiment M is the radical of 5,6-dimethylbenzimidazole.

In still another embodiment K is CH(OH).

In yet another embodiment E is O.

In another embodiment G^1 is OH.

In still another embodiment, all constituents of the conjugate assume their natural roles in vitamin B_{12}, except for the moieties through which any neutron capture agents are linked. The neutron capture agent(s) are preferably linked to the vitamin B_{12} structure through Z^2, Z^4 and/or Z^5, and even more preferably through the Z^2 moieties.

III. TC- or IF-Binding Carriers Comprising Gadolinium-157

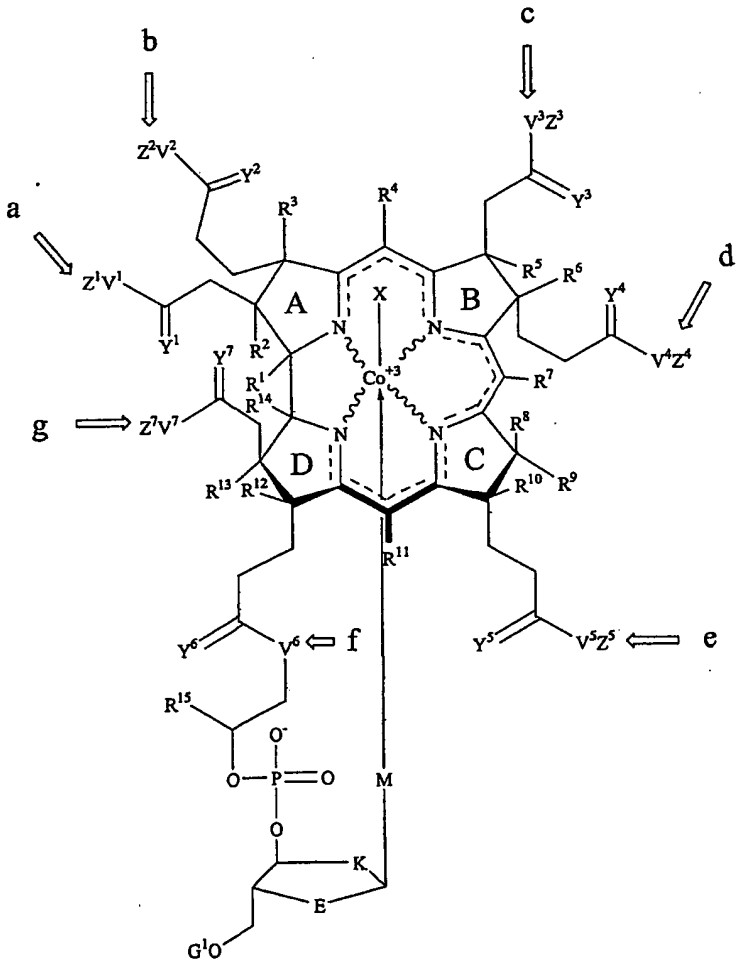
The metallic radionuclide Gadolinium-157 is an especially useful ion for conducting magnetic resonance imaging. It is also a useful target ion for neutron capture therapy. Applicants have discovered that incorporation of Gd-157 into the cobalamin conjugate of the present invention (wherein the Gd-157 supplements or replaces B-10) provides a compound that is not only particularly useful for conducting magnetic resonance imaging, but also a compound that can be used in conjunction with neutron capture therapy, to treat tumors.

Thus, in another embodiment the invention provides a method of performing neutron capture therapy using a Gd-157 conjugate having a high specificity for abnormally proliferative cells, or an enantiomer, diastereomer, salt or pro-drug thereof, comprising (1) a TC- or IF-binding carrier, and (2) a Gd-157 molecule linked directly or through a linker to the carrier. The method comprises administering the Gd-157 conjugate to a mammal (preferably a human), and initiating neutron capture.

Again, suitable ligands may be ascertained using any one of several means known in the art, including competitive binding assays with the receptor modulating agent competing with native vitamin B_{12}. An unsaturated Vitamin B12 binding capacity (UBBC) assay to assess the *in vitro* binding of the conjugate to the transcobalamin proteins is described by D. A. Collins and H. P. C. Hogenkamp in *J. Nuclear Medicine,* 1997, *38*, 717-723. See also Fairbanks, V. F. *Mayo Clinical Proc.* 83, Vol 58, 203-204.

The neutron capture agent is preferably bound directly or indirectly through an amide residue at the b-position, as illustrated in **Figure 1.**

In one embodiment the Gd-157 conjugate is a compound of formula **(II)**, or an enantiomer, diastereomer, salt or pro-drug thereof:



(II)

or its enantiomer, diastereomer or the pharmaceutically acceptable salt or prodrug thereof, wherein:

(i) the wavy line in the chemical structure indicates either a dative or covalent bond such that there are three dative Co-N bonds and one covalent Co-N bond, wherein, in the case of the dative bond, the valance of nitrogen is completed either with a double bond with an adjacent ring carbon or with a hydrogen;

(ii) the dotted line in the chemical structure indicates either a double or single bond such that the double bond does not over-extend the valence of the element (i.e. to give pentavalent carbons) and, in the case of a single bond, the valence is completed with hydrogen; and preferably, the bonding and stereochemistry of the compound is the same as that of vitamin B_{12} as it exists in nature;

(iii) X is hydrogen, cyano, halogen (Cl. F, Br or I), haloalkyl (including CF_3, CF_2CF_3, CH_2CF_3 and CF_2Cl), NO_2, NO_3, $PR^{15}R^{16}R^{17}$, NH_2, $NR^{15}R^{16}$, OH, OR^{15}, SR^{15}, SCN, N_3, $OC(O)R^{15}$, $C(O)_2R^{15}$, $C(O)R^{15}$, $OC(O)NR^{15}R^{16}$, $C(O)_2NR^{15}R^{16}$, $C(O)NR^{15}R^{16}$, $P(O)_2OR^{15}$, $S(O)_2OR^{15}$, a purine or pyrimidine nucleoside or nucleoside analog, including adenosyl (preferably linked through a 5'-deoxy linkage), alkyl, alkenyl, alkynyl, aryl, aralkyl, alkaryl, amino acid, peptide, protein, carbohydrate, heteroalkyl, heterocycle, heteroaryl, or alkylheteroaryl; alternatively, in one embodiment that is less preferred, X is L-T;

(iv) M is a monovalent heterocycle or heteroaromatic, which is capable of binding to the adjacent sugar ring and forming a dative bond with Co^{+3}, and is preferably a benzimidazole, a 5- and/or 6- substituted benzimidazole, such as 5,6-dimethylbenzimidazole, 5-methyl-benzimidazole, 5-hydroxybenzimidazole, 5-methoxy-benzimidazole, naphth-imidazole, 5-hydroxy-6-methyl-benzimidazole or 5-methoxy-6-methyl-benz-imidazole; or a purine or pyrimidine including but not limited to adenine, 2-methyladenine, 2-methylmercaptoadenine, e-methylsulfinyladenine, 2-methyl-sulfonyladenine and guanine; or a phenol, such as phenol or p-cresol;

(v) K is O, S, NJ^1, C(OH)H, $CR^{100}R^{101}$ or $C(R^{100})V^8Z^8$;

(vi) E is O or S;

(vii) G^1 is hydrogen, alkyl, acyl, silyl, phosphate or L-T;

(viii) Y^1, Y^2, Y^3, Y^4, Y^5, Y^6 and Y^7 independently are O, S or NJ^2;

(ix) V^1, V^2, V^3, V^4, V^5, V^6, V^7 and V^8 independently are O, S, NJ^3, $CR^{102}R^{103}$ or a direct bond;

(x) Z^1, Z^2, Z^3, Z^4, Z^5, Z^7 and Z^8 independently are R^{104} or L-T;

(xi) each L is independently a direct bond or a linker to one or more T moieties, and that does not significantly impair the ability of the TC- or IF- binding carrier to bind to a transcobalamin receptor, optionally via a transport protein;

(xii) each T independently comprises the residue of one or more molecules comprising Gd-157 or one or more radionuclides bound to L through a chelating moiety (in one embodiment T is for the treatment of a proliferative disorder other than cancer);

(xiii) at least one of Z^1, Z^2, Z^3, Z^4, Z^5, Z^7, Z^8, K and G^1 is L-T (in a preferred embodiment, Z^2 comprises the sole L-T in the TC- or IF- binding carrier);

(xiv) J^1, J^2 and J^3 independently are hydrogen, alkyl, alkenyl, alkynyl, alkaryl, cycloalkyl, aryl, cycloaryl, heteroalkyl, heterocycle, heteroaryl, hydroxyl, alkoxy or amine;

(xv) R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{12}, R^{13}, and R^{14} independently are hydrogen, lower alkyl, lower alkenyl, lower alkynyl, lower cycloalkyl, heteroalkyl, heterocyclic, lower alkoxy, azido, amino, lower alkylamino, halogen, thiol, SO_2, SO_3, carboxylic acid, C_{1-6} carboxyl, hydroxyl, nitro, cyano, oxime or hydrazine;

(xvi) R^{13} and R^{14} optionally can form a double bond;

(xvii) R^{15}, R^{16} and R^{17} are independently hydrogen, alkyl, alkenyl, alkynyl, aryl, alkaryl or aralkyl group, heteroalkyl, heterocycle or heteroaromatic; and

(xviii) R^{100}, R^{101}, R^{102}, R^{103}, and R^{104} are independently hydrogen, alkyl, alkenyl, alkynyl, aryl, acyl, heteroaromatic, heteroaryl, heteroalkyl, hydroxyl, alkoxy, cyano, azido, halogen, nitro, SO_2, SO_3, thioalkyl, or amino.

Preferred and more specific embodiments of the Gd-157 conjugate are the same as those set forth above for neutron capture agents such as B-10.

IV. Linker

The TC- or IF-binding carrier and neutron capture agent useful to treat and optionally image sites of proliferative disease in the body, such as cancerous tumors, can be combined by means of a di- or multi-valent linking moiety. The linker used to join the TC binding carrier and the neutron capture agent preferably has a single molecular weight, and does not exhibit a molecular weight distribution, for example as found in most polymers. The linker can range in size from small to large molecular weight, as long as there is not a distribution of weights in the linker. It is important to strictly control the uniformity of size of the conjugate for predictability of therapeutic performance.

As noted above, L is the residue of a linker molecule that conjugates one or more neutron capture agents to the TC ligand. The structure of the linker from which L is derived (in any one of the Z^1, Z^2, Z^3, Z^4, Z^5, Z^6, Z^7, X, M, K or G^1 moieties) is not crucial, provided it does not significantly impair the ability of the conjugate to bind to the transcobalamin or IF transport protein or receptor. L is preferably any multivalent molecule (divalent or greater) that does not significantly impair the ability of the TC carrier to bind to the transcobalamin transport protein or receptor. The ability of vitamin B_{12} or any other TC-binding or IF-binding carrier to bind to the transcobalamin transport protein or receptor is "significantly impaired" when attaching a linking moiety to the B_{12} or TC-binding or IF-binding carrier lessens the affinity of the vitamin B_{12} or the TC-binding or IF-binding carrier for the transcobalamin transport protein to which the vitamin B_{12} or TC-binding or IF-binding carrier is most readily bound by 50% or more. The unsaturated vitamin B_{12} binding capacity (UBBC) assay described by D. A. Collins and H. P. C. Hogenkamp in *J. Nuclear Medicine,* **1997**, *38*, 717-723 can be used to compare the relative affinities of ligands for this receptor .

In one embodiment the linker is of precise molecular weight and does not posses a molecular weight distribution. In one embodiment, the linker has a molecular weight less than about 2,500, 2,000, 1900, 1800, 1,500, 1,000 or 500. The linkers preferably have a

molecular weight below about 2000, more preferably below about 1000, and even more preferably below about 500.

A particularly preferred linker is one having multiple sites for conjugation to one or more neutron capture agents, wherein the linker has a unimodal molecular weight. Recombinant protein production techniques can be employed to obtain poly(amino acid) linkers of substantially constant molecular weight.

In one embodiment the linker is an amino acid, or a polymer or peptide formed from a plurality of amino acids. The polymer or peptide can be derived from one or more amino acids. The amino acid, poly(amino acid) or peptide can link T to V through the carboxy terminus or the amino terminus. The amino acid residue, peptide residue, or poly(amino acid) residue can conveniently be linked to V and T through an amide (e.g., -N(R)C(-O)- or –C(=O)N(R)-), ester (e.g., -OC(=O)- or -C(=O)O-), ether (e.g., -O-), ketone (e.g., -C(=O)-), thioether (e.g., -S-), sulfinyl (e.g., -S(O)-), sulfonyl (e.g., $-S(O)_2-$), or a direct (e.g., C-C bond) linkage, wherein each R is independently H or (C_1-C_{14}) alkyl.

Peptide derivatives can be prepared as disclosed in U.S. Patent Numbers 4,612,302; 4,853,371; and 4,684,620. Peptide sequences specifically recited herein are written with the amino terminus on the left and the carboxy terminus on the right, but are meant to also include the opposite flow. Particularly suitable peptides and poly(amino acids) comprise from 2 to about 20 amino acids, from 2 to about 15 amino acids, or from 2 to about 12 amino acids.

One exemplary poly(amino acid) is poly-L-lysine ($(-NHCH((CH_2)_4-NH_2)CO-)_m$-Q, wherein Q is H, ($C_1$-$C_{14}$)alkyl, or a suitable carboxy protecting group, and m is from 2 to about 20, from about 5 to about 15, or from about 8 to about 11. The polylysine offers multiple primary amine sites to which anti-proliferative agents can be readily attached. Alternatively, the linkers can be formed with multiple cysteines, to provide free thiols, or multiple glutamates or aspartates, to provide free carboxyls for conjugation using suitable carbodiimides. Similarly the linker can contain multiple histidines or tyrosines for conjugation. Other exemplary poly(amino acid) linkers are poly-L-glutamic acid, poly-L-aspartic acid, poly-L-histidine, poly-L-ornithine, poly-L-serine, poly-L-threonine, poly-L-tyrosine, poly-L-lysine-L-phenylalanine or poly-L-lysine-L-tyrosine. When the linker is derived from a poly(amino acid) other than polylysine, the linker is, in a series of

embodiments, prepared from 2 to about 30 amino acids, 5 to about 20 amino acids, or 8 to about 15 amino acids.

In another particular embodiment L is a polyamine residue (having at least three amino moieties) of the following chemical structure: NR'(alkylene-NR')$_n$alkyleneNR', wherein n is from 1 to 20, the carbon length of alkylene can vary within the n units, and each R' is independently hydrogen, lower alkyl, or T. N is preferably from 1 to 10. Moreover, L preferably has a backbone along its longest length of no more than 100, 75, 50, 40, 30, 20 or 15 atoms. Exemplary polyamines from which L can be derived include spermine ($H_2N(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$), spermidine ($H_2N(CH_2)_3NH(CH_2)_4NH_2$), decamethylene tetraamine, and pentamethylene hexamine. These linkers are a definite size and thus provide consistent and predictable targeting by the cobalamin conjugate, in addition to multiple binding sites for the neutron capture agent.

In another embodiment L is a diamine represented by the formula NH_2 $(CH_2)_x$ NH_2, in which x is 2-20, and preferably 2-12. Thus, the linker can be prepared from 1,6-diaminohexane, 1,5-diaminopentane, 1,4-diaminobutane and 1,3-diaminopropane.

Other suitable linkers are formed from the covalent linkage of various water soluble molecules with amino acids, peptides, poly(amino acids), polyamines, polyoxyalkylenes, polyanhydrides, polyesters, polyamides, polyglycolides and diamines. Suitable water soluble molecules include, for example, polyethylene glycol, and dicarboxylic monosaccharides such as glucaric acid, galactaric acid and xylaric acid.

Other suitable linkers include those represented by the formula $HO(O)C(CH_2)_xC(O)OH$, in which x is 2-20, and preferably 2-12. Thus, the linker can be prepared from succinic acid, glutaric acid, adipic acid, suberic acid, sebacic acid, azelaic acid or maleic acid. Still other suitable linkers comprise carboxylic acid derivatives that yield an amide upon reaction with an amine. Such reactive groups include, by way of example, carboxylic acid halides such as acid chlorides and bromides; carboxylic acid anhydrides such as acetic anhydrides and trifluoroacetic anhydrides; esters such as p-nitrophenyl esters and N-hydroxysuccinimide esters; and imidazolides. Techniques for using such linkers are described in detail in Bodanszky, Principles of Peptide Synthesis, Springer Verlag, Berlin, 1984.

In one embodiment, the linker is modified to facilitate its conjugation either to V or T. Suitable molecules for modifying the linker include: disuccinimidyl suberate (DSS),

bis(sulfosuccinimidyl) suberate (BSS), ethylene glycolbis(succinimidylsuccinate) (EGS), ethylene glycolbis(sulfosuccinimidyl-succinate) (Sulfo-EGS), p-aminophenylacetic acid, dithiobis(succinimidyl-propionate) (DSP), 3,3'-dithiobis-(sulfosuccinimidylpropionate) (DTSSP), disuccinimidyl tartarate (DST), disulfosuccinimidyl tartarate (Sulfo-DST), bis-(2-(succinimidooxycarbonyloxy)-ethylene)sulfone (BSOCOES), bis(2-(sulfosuccinimido-oxycarbonyloxy)ethylene)sulfone (Sulfo-BSOCOES), dimethyl adipimidate 2HCl (DMA), dimethyl pimelimidate 2HCl (DMP), and dimethyl suberimidate 2HCl (DMS).

Biodegradable linkers

Various degradable linkers can be used to link the TC-binding or IF-binding moiety to the neutron capture agent. The desired linkers can degrade under biological conditions such as by enzymatic cleavage or by systemic pH or temperature. Alternatively, these linkers can be induced to degrade by external manipulation such as changes in pH, temperature, ultrasound, magnetic field, radiation (i.e. UV radiation) or light.

U.S. Patent No. 5,639,885 entitled "Redox amino acids and peptides containing them;" U.S. Patent No. 5,637,601 entitled "Anticholinergic compounds, compositions and methods of treatment;" U.S. Patent No. 5,624,894 entitled "Brain-enhanced delivery of neuroactive peptides by sequential metabolism;" U.S. Patent No. 5,618,826 entitled "Anticholinergic compounds, compositions and methods of treatment;" U.S. Patent No. 5,618,803 entitled "Targeted drug delivery via phosphonate derivatives;" U.S. Patent No. 5,610,188 entitled "Anticholinergic compounds, compositions and methods of treatment;" U.S. Patent No. 5,525,727 entitled "Brain-specific drug delivery;" U.S. Patent No. 5,418,244 entitled "Anticholinergic compounds, compositions and methods of treatment;" U.S. Patent No. 5,413,996 entitled "Targeted drug delivery via phosphonate derivatives;" U.S. Patent No. 5,389,623 entitled "Redox carriers for brain-specific drug delivery;" U.S. Patent No. 5,296,483 entitled "Brain-specific analogues of centrally acting amines;" U.S. Patent No. 5,258,388 entitled "Anticholinergic compounds, compositions and methods of treatment;" U.S. Patent No. 5,231,089 entitled "Method of improving oral bioavailability of carbamazepine;" U.S. Patent No. 5,223,528 entitled "Anticholinergic compounds, compositions and methods of treatment;" U.S. Patent No. 5,187,158 Brain-specific drug

delivery;" U.S. Patent No. 5,177,064 entitled "Targeted drug delivery via phosphonate derivatives;" U.S. Patent No. 5,155,227 entitled "Compounds for site-enhanced delivery of radionuclides;" U.S. Patent No. 5,136,038 entitled "Radiopharmaceuticals and chelating agents useful in their preparation;" U.S. Patent No. 5,087,618 entitled "Redox carriers for brain-specific drug delivery;" U.S. Patent No. 5,079,366 entitled "Quaternary pyridinium salts;" U.S. Patent No. 5,053,215 entitled "NMR-assayable ligand-labeled trifluorothymidine containing composition and method for diagnosis of HSV infection;" U.S. Patent No. 5,024,998 entitled "Pharmaceutical formulations for parenteral use;" U.S. Patent No. 5,017,618 entitled "Labile derivatives of ketone analogs of 3-substituted-1-alkylamino-2-propanols and their use as beta-adrenergic blockers;" U.S. Patent No. 5,017,566 entitled "Redox systems for brain-targeted drug delivery;" U.S. Patent No. 5,008,257 entitled "Brain-specific drug delivery;" U.S. Patent No. 5,002,935 entitled "Improvements in redox systems for brain-targeted drug delivery;" U.S. Patent No. 4,983,586 entitled "Pharmaceutical formulations for parenteral use;" U.S. Patent No. 4,963,688 entitled "Compounds for site-enhanced delivery of radionuclides and uses thereof;" U.S. Patent No. 4,963,682 entitled "Novel radiopharmaceuticals and chelating agents useful in their preparation;" U.S. Patent No. 4,933,438 entitled "Brain-specific analogues of centrally acting amines;" U.S. Patent No. 4,900,837 entitled "Brain-specific drug delivery of steroid sex hormones cleaved from pyridinium carboxylates and dihydropyridine carboxylate precursors;" U.S. Patent No. 4,892,737 entitled "Composition and method for enhancing permeability of topical drugs;" U.S. Patent No. 4,888,427 entitled "Amino acids containing dihydropyridine ring systems for site-specific delivery of peptides to the brain;" 4,880,921 entitled "Brain-specific drug delivery;" 35. 4,863,911 entitled "Method for treating male sexual dysfunction;" U.S. Patent No. 4,829,070 entitled "Novel redox carriers for brain-specific drug delivery;" U.S. Patent No. 4,824,850 entitled "Brain-specific drug delivery;" U.S. Patent No. 4,801,597 entitled "Certain inositol-nicotinate ester derivatives and polyionic complexes therefore useful for treating diabetes meuitus, hyperlipidemia and lactic acidosis;" U.S. Patent No. 4,771,059 entitled "Brain-specific analogues of centrally acting amines;" U.S. Patent No. 4,727,079 entitled "Brain-specific dopaminergic activity involving dihydropyridine carboxamides, dihydroquinoline and isoquinoline carboxamides;" U.S. Patent No. 4,540,564 entitled "Brain-specific drug delivery;" and U.S. Patent No. 4,479,932 entitled "Brain-specific drug delivery" to Nicholas S. Bodor, et al., disclose several biodegradable linkers that target the brain. For example, a lipoidal form of dihydropyridine pyridinium salt redox carrier, DHC, linked to

a centrally acting drug which can be reduced and biooxidized to pass through the blood brain barrier. The dihydropyridine nucleus readily and easily penetrates the blood brain barrier in increased concentrations; furthermore, the in vivo oxidation of the dihydropyridine moiety to the ionic pyridinium salts thereby prevents its elimination from the brain, while elimination from the general circulation is accelerated, resulting in a prolongedly sustained brain-specific drug activity. This dihydropyridine can be incorporated into the linkers set forth above for biodegradation.

Additionally U.S. Patent No. 4,622,218 entitled "Testicular-specific drug delivery," discloses linkers that can specifically deliver drugs to the testes in much the same manner, and which can be used in the linkers of the present invention. The lipoidal form [D--DHC] of a dihydropyridine pyridinium salt redox carrier, e.g. 1,4-dihydrotrigonelline, penetrates the blood-testis barrier. Oxidation of the dihydropyridine carrier moiety in vivo to the ionic pyridinium salt type drug/carrier entity $[D\text{--}QC]^+$ prevents elimination thereof from the testes, while elimination from the general circulation is accelerated, resulting in significant and prolongedly sustained testicular-specific drug activity.

Margerum, *et al.* in U.S. Patent No. 5,976,493 discloses the use of polychelant compounds which are degradable *in vivo* to release excretable fragments for diagnostic imaging which also are suitable in the linkers of the present invention. These compounds contain a linker moiety which is metabolically cleavable to release macrocyclic monochelant fragments, wherein the macrocyclic skeleton preferably has 9 to 25 ring members, and a biotolerable polymer, preferably a substantially monodisperse polymer. Other suitable linkers are disclosed, for example, in Krejcarek et al. (Biochemical and Biophysical Research Communications 77: 581 (1977)) (mixed anhydrides), Hnatowich et al. (Science 220: 613 (1983))(cyclic anhydrides), United States Patent 5,637,684 to Cook, *et al.* (Phosphoramidate and phosphorothioamidate oligomeric compounds).

Other suitable biodegradable polymers from which the linker can be formed are the polyanhydrides and polyorthoesters, which take advantage of labile backbone linkages (see: Domb et al. Macromolecules, *22*, 3200, **1989**; and Heller et al. Biodegradable Polymers as Drug Delivery Systems, Dekker, NY: **1990**). Other linker materials include hydrogels, such as the PEG-oligoglycolyl-acrylates disclosed in U.S. Patent No. 5,626,863 to Hubbell et al.. Other biodegradable linkers are formed from oligoglycolic acid is a

poly(a-hydroxy acid), polylactic acid, polycaprolactone, polyorthoesters, polyanhydrides and polypeptides.

Nonlimiting examples of U.S. Patents that describe controlled release formulations suitable for use as linking agents are: U.S. Patent No. 5,356,630 to Laurencin *et al.* (Delivery System for Controlled Release of Bioactive Factors); U.S. Patent No. 5,797,898 to Santini, Jr. *et al.* (Microchip Drug Delivery Devices); U.S. Patent No. 5,874,064 to Edwards *et al.* (Aerodynamically Light Particles for Pulmonary Drug Delivery); U.S. Patent No. 5,548,035 to Kim *et al.* (Biodegradable Copolymer as Drug Delivery Matrix Comprising Polyethyleneoxide and Aliphatic Polyester Blocks); U.S. Patent No. 5,532,287 to Savage *et al.* (Radiation Cured Drug Release Controlling Membrane); U.S. Patent No. 5,284,831 to Kahl *et al.* (Drug Delivery Porphyrin Composition and Methods); U.S. Patent No. 5,741,329 to Agrawal *et al.* (Methods of Controlling the pH in the Vicinity of Biodegradable Implants); U.S. Patent No. 5,820,883 to Tice *et al.* (Methods for Delivering Bioactive Agents into and Through the Mucosally-Associated Lymphoid Tissues and Controlling Their Release);U.S. Patent No. 5,955,068 to Gouin *et al.* (Biodegradable polyanhydrides Derived from Dimers of Bile Acids, and Use Thereof as Controlled Drug Release Systems); U.S. Patent No. 6,001,395 to Coombes *et al.* (Polymeric Lamellar Substrate Particles for Drug Delivery); U.S. Patent No. 6,013,853 to Athanasiou *et al.* (Continuous Release Polymeric Implant Carriers); U.S. Patent No. 6,060,582 to Hubbell *et al.* (Photopolymerizable Biodegradable Hydrogels as Tissue Contacting Materials and Controlled Release Carriers); U.S. Patent No. 6,113,943 to Okada *et al.* (Sustained-Release Preparation Capable of Releasing a Physiologically Active Substance); and PCT Publication No. WO 99/59548 to Oh *et al.* (Controlled Drug Delivery System Using the Conjugation of Drug to Biodegradable Polyester); U.S. Patent No. 6,123,861 (Fabrication of Microchip Drug Delivery Devices); U.S. Patent No. 6,060,082 (Polymerized Liposomes Targeted to M cells and Useful for Oral or Mucosal Drug Delivery); U.S. Patent No. 6,041,253 (Effect of Electric Field and Ultrasound for Transdermal Drug Delivery); U.S. Patent No. 6,018,678 (Transdermal protein delivery or measurement using low-frequency sonophoresis); U.S. Patent No. 6,007,845 Nanoparticles And Microparticles Of Non-Linear Hydrophilic-Hydrophobic Multiblock Copolymers; U.S. Patent No. 6,004,534 Targeted Polymerized Liposomes For Improved Drug Delivery; U.S. Patent No. 6,002,961 Transdermal Protein Delivery Using Low-Frequency Sonophoresis; U.S. Patent No. 5,985,309 Preparation Of Particles For

Inhalation; U.S. Patent No. 5,947,921 Chemical And Physical Enhancers And Ultrasound For Transdermal Drug Delivery; U.S. Patent No. 5,912,017 Multiwall Polymeric Microspheres; U.S. Patent No. 5,911,223 Introduction Of Modifying Agents Into Skin By Electroporation; U.S. Patent No. 5,874,064 Aerodynamically Light Particles For Pulmonary Drug Delivery; U.S. Patent No. 5,855,913 Particles Incorporating Surfactants For Pulmonary Drug Delivery; U.S. Patent No. 5,846,565 Controlled Local Delivery Of Chemotherapeutic Agents For Treating Solid Tumors; U.S. Patent No. 5,837,752 Semi-Interpenetrating Polymer Networks; U.S. Patent No. 5,814,599 Transdermal Delivery Of Encapsulated Drugs; U.S. Patent No. 5,804,178 Implantation Of Cell-Matrix Structure Adjacent Mesentery, Omentum Or Peritoneum Tissue; U.S. Patent No. 5,797,898 Microchip Drug Delivery Devices; U.S. Patent No. 5,770,417 Three-Dimensional Fibrous Scaffold Containing Attached Cells For Producing Vascularized Tissue In Vivo; U.S. Patent No. 5,770,193 Preparation Of Three-Dimensional Fibrous Scaffold For Attaching Cells To Produce Vascularized Tissue In Vivo; U.S. Patent No. 5,762,904 Oral Delivery Of Vaccines Using Polymerized Liposomes; U.S. Patent No. 5,759,830 Three-Dimensional Fibrous Scaffold Containing Attached Cells For Producing Vascularized Tissue In Vivo; U.S. Patent No. 5,749,847 Delivery Of Nucleotides Into Organisms By Electroporation; U.S. Patent No. 5,736,372 Biodegradable Synthetic Polymeric Fibrous Matrix Containing Chondrocyte For In Vivo Production Of A Cartilaginous Structure; U.S. Patent No. 5,718,921 Microspheres Comprising Polymer And Drug Dispersed There Within; U.S. Patent No. 5,696,175 Preparation Of Bonded Fiber Structures For Cell Implantation; U.S. Patent No. 5,667,491 Method For Rapid Temporal Control Of Molecular Transport Across Tissue; U.S. Patent No. 5,654,381 Functionalized Polyester Graft Copolymers; U.S. Patent No. 5,651,986 Controlled Local Delivery Of Chemotherapeutic Agents For Treating Solid Tumors; U.S. Patent No. 5,629,009 Delivery System For Controlled Release Of Bioactive Factors; U.S. Patent No. 5,626,862 Controlled Local Delivery Of Chemotherapeutic Agents For Treating Solid Tumors; U.S. Patent No. 5,593,974 Localized Oligonucleotide Therapy; U.S. Patent No. 5,578,325 Nanoparticles And Microparticles Of Non-Linear Hydrophilic-Hydrophobic Multiblock Copolymers; U.S. Patent No. 5,562,099 Polymeric Microparticles Containing Agents For Imaging; U.S. Patent No. 5,545,409 Delivery System For Controlled Release Of Bioactive Factors; U.S. Patent No. 5,543,158 Biodegradable Injectable Nanoparticles; U.S. Patent No. 5,514,378 Biocompatible Polymer Membranes And Methods Of Preparation Of Three Dimensional Membrane Structures; U.S. Patent No. 5,512,600

Preparation Of Bonded Fiber Structures For Cell Implantation; U.S. Patent No. 5,500,161 Method For Making Hydrophobic Polymeric Microparticles; U.S. Patent No. 5,487,390 Gas-filled polymeric microbubbles for ultrasound imaging; U.S. Patent No. 5,399,665 Biodegradable polymers for cell transplantation; U.S. Patent No. 5,356,630 Delivery system for controlled release of bioactive factors; U.S. Patent No. 5,330,768 Controlled drug delivery using polymer/pluronic blends; U.S. Patent No. 5,286,763 Bioerodible polymers for drug delivery in bone; U.S. Patent No. 5,149,543 Ionically cross-linked polymeric microcapsules; U.S. Patent No. 5,128,420 Method of making hydroxamic acid polymers from primary amide polymers; U.S. Patent No. 5,122,367 Polyanhydride bioerodible controlled release implants for administration of stabilized growth hormone; U.S. Patent No. 5,100,668 Controlled release systems containing heparin and growth factors; U.S. Patent No. 5,019,379 Unsaturated polyanhydrides; U.S. Patent No. 5,010,167 Poly(amide-and imide-co-anhydride) for biological application; .S. Patent No. 4,948,587 Ultrasound enhancement of transbuccal drug delivery; U.S. Patent No. 4,946,929 Bioerodible articles useful as implants and prostheses having predictable degradation rates; U.S. Patent No. 4,933,431 One step preparation of poly(amide-anhydride); U.S. Patent No. 4,933,185 System for controlled release of biologically active compounds; U.S. Patent No. 4,921,757 System for delayed and pulsed release of biologically active substances; U.S. Patent No. 4,916,204 Pure polyanhydride from dicarboxylic acid and coupling agent; U.S. Patent No. 4,906,474 Bioerodible polyanhydrides for controlled drug delivery; U.S. Patent No. 4,900,556 System for delayed and pulsed release of biologically active substances; U.S. Patent No. 4,898,734 Polymer composite for controlled release or membrane formation; U.S. Patent No. 4,891,225 Bioerodible polyanhydrides for controlled drug delivery; U.S. Patent No. 4,888,176 Controlled drug delivery high molecular weight polyanhydrides; .S. Patent No. 4,886,870 Bioerodible articles useful as implants and prostheses having predictable degradation rates; U.S. Patent No. 4,863,735 Biodegradable polymeric drug delivery system with adjuvant activity; U.S. Patent No. 4,863,611 Extracorporeal reactors containing immobilized species; U.S. Patent No. 4,861,627 Preparation of multiwall polymeric microcapsules; U.S. Patent No. 4,857,311 Polyanhydrides with improved hydrolytic degradation properties; U.S. Patent No. 4,846,786 Bioreactor containing suspended, immobilized species; U.S. Patent No. 4,806,621 Biocompatible, bioerodible, hydrophobic, implantable polyimino carbonate article; U.S. Patent No. 4,789,724 Preparation of anhydride copolymers; U.S. Patent No. 4,780,212 Ultrasound enhancement

of membrane permeability; U.S. Patent No. 4,779,806 Ultrasonically modulated polymeric devices for delivering compositions; U.S. Patent No. 4,767,402 Ultrasound enhancement of transdermal drug delivery; U.S. Patent No. 4,757,128 High molecular weight polyanhydride and preparation thereof; .S. Patent No. 4,657,543 Ultrasonically modulated polymeric devices for delivering compositions; U.S. Patent No. 4,638,045 Non-peptide polyamino acid bioerodible polymers; U.S. Patent No. 4,591,496 Process for making systems for the controlled release of macromolecules.

Nonmetallic radioisotopes can conveniently be linked to the vitamin B_{12} structure through a residue of a peptide having the following formula:

wherein each M is independently a non-metallic radionuclide; each R is independently (C_1-C_{14})alkyl, (C_2-C_{14})alkenyl, (C_2-C_{14})alkynyl, (C_1-C_{14})alkoxy, hydroxy, cyano, nitro, halo, trifluoromethyl, N(R_a)(R_b), (C_1-C_{14})alkanoyl, (C_2-C_{14})alkanoyloxy, (C_6-C_{10})aryl, or (C_3-C_8)cycloalkyl wherein R_a and R_b are each independently H or (C_1-C_{14})alkyl; P; Q is H, (C_1-C_{14})alkyl, or a suitable carboxy protecting group; n is 2 to about 20; I is 1-5, j is 0-4 and I+j is ≤ 5; or a pharmaceutically acceptable salt thereof. Specifically, i can be 1, j can be 0, M can be a positron emitter such as Fluorine-18, Bromine-76, Iodine-124 or a gamma emitter such as Iodine-123 or Iodine-131, and n can be about 6 to about 12.

The above discussion has demonstrated how the various variables associated with the cobalamin conjugates of the present invention can be independently varied to more particularly define specific classes of cobalamin conjugates encompassed by this invention. It is to be understood that the modification of one variable can be made independently of the modification of any other variable. Moreover, any number of embodiments can be defined by modifying two or more of the variables in such embodiments. A few of such embodiments are described below for purposes of exemplification.

Subembodiment 1: X is 5'-deoxyadenosyl; M is a monovalent heterocycle that is capable of binding to the adjacent sugar ring, and forming a dative bond with Co^{+3}, optionally substituted by L-T; K is O, S, NJ^1, $CR^{100}R^{101}$, or $C(R^{100})V^8Z^8$; E is O or S; G^1 is

hydrogen, alkyl, acyl, silyl, phosphate, or L-T; Y^1, Y^2, Y^3, Y^4, Y^5, Y^6 and Y^7 independently are O, S or NJ^2; V^1, V^2, V^3, V^4, V^5, V^6, V^7 and V^8 independently are O, S or NJ^3; $CR^{102}R^{103}$, or a direct bond; Z^1, Z^2, Z^3, Z^4, Z^5, Z^7 and Z^8 independently are R^{104} or L-T; each L is independently a direct bond or the residue of a multivalent moiety that does not significantly impair the ability of the compound to bind transcobalamin II; each T independently comprises the residue of one or more molecules comprising B-10 or one or more radionuclides; at least one of Z^1, Z^2, Z^3, Z^4, Z^5, Z^7 and Z^8, M, K, E or G^1 comprises a molecule comprising B-10; J^1, J^2 and J^3 independently are hydrogen, alkyl, alkenyl, alkynyl, alkaryl, cycloalkyl, aryl, cycloaryl, heterocycle, heteroaryl, hydroxyl, alkoxy, or amine; R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{12}, R^{13}, R^{14} and R^{15} retain their natural vitamin B_{12} configuration; and R^{100}, R^{101}, R^{102}, R^{103}, and R^{104} are independently hydrogen, alkyl, alkenyl, alkynyl, hydroxyl, alkoxy, cyano, azido, halogen, nitro, SO_2, SO_3, thioalkyl, or amino.

Subembodiment 2: X is 5'-deoxyadenosyl; M, K, E and G^1 retain their natural vitamin B12 configuration; Y^1, Y^2, Y^3, Y^4, Y^5, Y^6 and Y^7 independently are O, S or NJ^2; V^1, V^2, V^3, V^4, V^5, V^6, V^7 and V^8 independently are O, S or NJ^3; $CR^{102}R^{103}$, or a direct bond; Z^1, Z^2, Z^3, Z^4, Z^5, Z^7 and Z^8 independently are R^{104} or L-T; each L is independently a direct bond or the residue of a multivalent moiety that does not significantly impair the ability of the compound to bind transcobalamin II; each T independently comprises the residue of one or more molecules comprising B-10 or one or more radionuclides; at least one of Z^1, Z^2, Z^3, Z^4, Z^5, Z^7 and Z^8, M, K, E, or G^1 comprises a molecule comprising B-10; J^1, J^2 and J^3 independently are hydrogen, alkyl, alkenyl, alkynyl, alkaryl, cycloalkyl, aryl, cycloaryl, heterocycle, heteroaryl, hydroxyl, alkoxy, or amine; R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{12}, R^{13}, R^{14} and R^{15} independently are hydrogen, lower alkyl, lower alkenyl, lower alkynyl, lower cycloalkyl, heterocyclic, lower alkoxy, azido, amino, lower alkylamino, halogen, thiol, SO_2, SO_3, carboxylic acid, $C_{1\text{-}6}$ carboxyl, hydroxyl, nitro, cyano, oxime or hydrazine; R^{13} and R^{14} optionally can come together to form a double bond; and R^{100}, R^{101}, R^{102}, R^{103}, and R^{104} are independently hydrogen, alkyl, alkenyl, alkynyl, hydroxyl, alkoxy, cyano, azido, halogen, nitro, SO_2, SO_3, thioalkyl, or amino.

Subembodiment 3: X is 5'-deoxyadenosyl; M is a monovalent heterocycle that is capable of binding to the adjacent sugar ring, and forming a dative bond with Co^{+3}, optionally substituted by L-T; K is O, S, NJ^1, $CR^{100}R^{101}$, or $C(R^{100})V^8Z^8$; E is O or S; G^1 is

hydrogen, alkyl, acyl, silyl, phosphate, or L-T; Y^1, Y^2, Y^3, Y^4, Y^5, Y^6 and Y^7 independently are O, S or NJ^2; V^1, V^2, V^3, V^4, V^5, V^6, V^7 and V^8 independently are O, S or NJ^3; $CR^{102}R^{103}$, or a direct bond; Z^1, Z^2, Z^3, Z^4, Z^5, Z^7 and Z^8 independently are R^{104} or L-T; each L is independently a direct bond or the residue of a multivalent moiety that does not significantly impair the ability of the compound to bind transcobalamin II; each T independently comprises the residue of one or more molecules comprising B-10 or one or more radionuclides; at least one of Z^2, Z^4, or Z^5 comprises a molecule comprising B-10, the remaining Z moieties retaining their natural vitamin B_{12} configuration; J^1, J^2 and J^3 independently are hydrogen, alkyl, alkenyl, alkynyl, alkaryl, cycloalkyl, aryl, cycloaryl, heterocycle, heteroaryl, hydroxyl, alkoxy, or amine; R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{12}, R^{13}, R^{14} and R^{15} independently are hydrogen, lower alkyl, lower alkenyl, lower alkynyl, lower cycloalkyl, heterocyclic, lower alkoxy, azido, amino, lower alkylamino, halogen, thiol, SO_2, SO_3, carboxylic acid, $C_{1\text{-}6}$ carboxyl, hydroxyl, nitro, cyano, oxime or hydrazine; R^{13} and R^{14} optionally can come together to form a double bond; and R^{100}, R^{101}, R^{102}, R^{103}, and R^{104} are independently hydrogen, alkyl, alkenyl, alkynyl, hydroxyl, alkoxy, cyano, azido, halogen, nitro, SO_2, SO_3, thioalkyl, or amino.

Subembodiment 4: X is hydrogen, cyano, amino, amido, hydroxyl, 5'-deoxyadenosyl, L-T, alkyl, alkenyl, alkynyl, cylcoalkyl, aryl, aralkyl, heterocycle, or heteroaryl, or alkylheteroaryl; M, K, E and G^1 retain their natural vitamin B_{12} configuration; Y^1, Y^2, Y^3, Y^4, Y^5, Y^6 and Y^7 independently are O, S or NJ^2; V^1, V^2, V^3, V^4, V^5, V^6, V^7 and V^8 independently are O, S or NJ^3; $CR^{102}R^{103}$, or a direct bond; Z^1, Z^2, Z^3, Z^4, Z^5, Z^7 and Z^8 independently are R^{104} or L-T; each L is independently a direct bond or the residue of a multivalent moiety that does not significantly impair the ability of the compound to bind transcobalamin II; each L is independently a direct bond or the residue of a multivalent moiety that does not significantly impair the ability of the compound to bind transcobalamin II; each T independently comprises the residue of one or more molecules comprising B-10 or one or more radionuclides; at least one of Z^1, Z^2, Z^3, Z^4, Z^5, Z^7 and Z^8, M, K, E or G^1 comprises a molecule comprising B-10; J^2 and J^3 independently are hydrogen, alkyl, alkenyl, alkynyl, alkaryl, cycloalkyl, aryl, cycloaryl, heterocycle, heteroaryl, hydroxyl, alkoxy, or amine; R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{12}, R^{13}, R^{14} and R^{15} retain their natural vitamin B_{12} configuration; and R^{100}, R^{101}, R^{102}, R^{103}, and R^{104} are independently hydrogen, alkyl, alkenyl, alkynyl, hydroxyl, alkoxy, cyano, azido, halogen, nitro, SO_2, SO_3, thioalkyl, or amino.

Subembodiment 5: X is hydrogen, cyano, amino, amido, hydroxyl, 5'-deoxyadenosyl, L-T, alkyl, alkenyl, alkynyl, cylcoalkyl, aryl, aralkyl, heterocycle, or heteroaryl, or alkylheteroaryl; B, K, E and G^1 retain their natural vitamin B_{12} configuration; Y^1, Y^2, Y^3, Y^4, Y^5, Y^6 and Y^7 independently are O, S or NJ^2; V^1, V^2, V^3, V^4, V^5, V^6, V^7 and V^8 independently are O, S or NJ^3; $CR^{102}R^{103}$, or a direct bond; Z^1, Z^2, Z^3, Z^4, Z^5, Z^7 and Z^8 independently are R^{104} or L-T; each L is independently a direct bond or the residue of a multivalent moiety that does not significantly impair the ability of the compound to bind transcobalamin II; each L is independently a direct bond or the residue of a multivalent moiety that does not significantly impair the ability of the compound to bind transcobalamin II; each T independently comprises the residue of one or more molecules comprising B-10 or one or more radionuclides; at least one of Z^2, Z^4, or Z^5 comprises a molecule comprising B-10, the remaining Z moieties retaining their natural vitamin B_{12} configuration; J^1, J^2 and J^3 independently are hydrogen, alkyl, alkenyl, alkynyl, alkaryl, cycloalkyl, aryl, cycloaryl, heterocycle, heteroaryl, hydroxyl, alkoxy, or amine; R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{12}, R^{13}, R^{14} and R^{15} independently are hydrogen, lower alkyl, lower alkenyl, lower alkynyl, lower cycloalkyl, heterocyclic, lower alkoxy, azido, amino, lower alkylamino, halogen, thiol, SO_2, SO_3, carboxylic acid, $C_{1\text{-}6}$ carboxyl, hydroxyl, nitro, cyano, oxime or hydrazine; R^{13} and R^{14} optionally can come together to form a double bond; and R^{100}, R^{101}, R^{102}, R^{103}, and R^{104} are independently hydrogen, alkyl, alkenyl, alkynyl, hydroxyl, alkoxy, cyano, azido, halogen, nitro, SO_2, SO_3, thioalkyl, or amino.

Subembodiment 6: X is hydrogen, cyano, amino, amido, hydroxyl, 5'-deoxyadenosyl, L-T, alkyl, alkenyl, alkynyl, cylcoalkyl, aryl, aralkyl, heterocycle, or heteroaryl, or alkylheteroaryl; M, K, E and G^1 retain their natural vitamin B_{12} configuration; Y^1, Y^2, Y^3, Y^4, Y^5, Y^6 and Y^7 independently are O, S or NJ^2; V^1, V^2, V^3, V^4, V^5, V^6, V^7 and V^8 independently are O, S or NJ^3; $CR^{102}R^{103}$, or a direct bond; Z^1, Z^2, Z^3, Z^4, Z^5, Z^7 and Z^8 independently are R^{104} or L-T; each L is independently a direct bond or the residue of a multivalent moiety that does not significantly impair the ability of the compound to bind transcobalamin II; each L is independently a direct bond or the residue of a multivalent moiety that does not significantly impair the ability of the compound to bind transcobalamin II; each T independently comprises the residue of one or more molecules comprising B-10 or one or more radionuclides; at least one of Z^2, Z^4, or Z^5 comprises a molecule comprising B-10, the remaining Z moieties retaining their natural

vitamin B_{12} configuration; J^1, J^2 and J^3 independently are hydrogen, alkyl, alkenyl, alkynyl, alkaryl, cycloalkyl, aryl, cycloaryl, heterocycle, heteroaryl, hydroxyl, alkoxy, or amine; R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{12}, R^{13}, R^{14} and R^{15} independently are hydrogen, lower alkyl, lower alkenyl, lower alkynyl, lower cycloalkyl, heterocyclic, lower alkoxy, azido, amino, lower alkylamino, halogen, thiol, SO_2, SO_3, carboxylic acid, C_{1-6} carboxyl, hydroxyl, nitro, cyano, oxime or hydrazine; R^{13} and R^{14} optionally can come together to form a double bond; and R^{100}, R^{101}, R^{102}, R^{103}, and R^{104} are independently hydrogen, alkyl, alkenyl, alkynyl, hydroxyl, alkoxy, cyano, azido, halogen, nitro, SO_2, SO_3, thioalkyl, or amino.

Subembodiment 7: X is 5'-deoxyadenosyl; M, K, E and G^1 retain their natural vitamin B_{12} configuration; Y^1, Y^2, Y^3, Y^4, Y^5, Y^6 and Y^7 independently are O, S or NJ^2; V^1, V^2, V^3, V^4, V^5, V^6, V^7 and V^8 independently are O, S or NJ^3; $CR^{102}R^{103}$, or a direct bond; Z^1, Z^2, Z^3, Z^4, Z^5, Z^7 and Z^8 independently are R^{104} or L-T; each L is independently a direct bond or the residue of a multivalent moiety that does not significantly impair the ability of the compound to bind transcobalamin II; each L is independently a direct bond or the residue of a multivalent moiety that does not significantly impair the ability of the compound to bind transcobalamin II; each T independently comprises the residue of one or more molecules comprising B-10 or one or more radionuclides; at least one of Z^1, Z^2, Z^3, Z^4, Z^5, Z^7 and Z^8, M, K, E or G^1 comprises a molecule comprising B-10; J^2 and J^3 independently are hydrogen, alkyl, alkenyl, alkynyl, alkaryl, cycloalkyl, aryl, cycloaryl, heterocycle, heteroaryl, hydroxyl, alkoxy, or amine; R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{12}, R^{13}, R^{14} and R^{15} retain their natural vitamin B_{12} configuration; and R^{100}, R^{101}, R^{102}, R^{103}, and R^{104} are independently hydrogen, alkyl, alkenyl, alkynyl, hydroxyl, alkoxy, cyano, azido, halogen, nitro, SO_2, SO_3, thioalkyl, or amino.

Subembodiment 8: X is 5'-deoxyadenosyl; M, K, E and G^1 retain their natural vitamin B_{12} configuration; Y^1, Y^2, Y^3, Y^4, Y^5, Y^6 and Y^7 independently are O, S or NJ^2; V^1, V^2, V^3, V^4, V^5, V^6, V^7 and V^8 independently are O, S or NJ^3; $CR^{102}R^{103}$, or a direct bond; Z^1, Z^2, Z^3, Z^4, Z^5, Z^7 and Z^8 independently are R^{104} or L-T; each L is independently a direct bond or the residue of a multivalent moiety that does not significantly impair the ability of the compound to bind transcobalamin II; each L is independently a direct bond or the residue of a multivalent moiety that does not significantly impair the ability of the compound to bind transcobalamin II; each T independently comprises the residue of one

or more molecules comprising B-10 or one or more radionuclides; at least one of Z^2, Z^4, or Z^5 comprises a molecule comprising B-10, the remaining Z moieties retaining their natural vitamin B_{12} configuration; J^1, J^2 and J^3 independently are hydrogen, alkyl, alkenyl, alkynyl, alkaryl, cycloalkyl, aryl, cycloaryl, heterocycle, heteroaryl, hydroxyl, alkoxy, or amine; R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{12}, R^{13}, R^{14} and R^{15} independently are hydrogen, lower alkyl, lower alkenyl, lower alkynyl, lower cycloalkyl, heterocyclic, lower alkoxy, azido, amino, lower alkylamino, halogen, thiol, SO_2, SO_3, carboxylic acid, $C_{1\text{-}6}$ carboxyl, hydroxyl, nitro, cyano, oxime or hydrazine; R^{13} and R^{14} optionally can come together to form a double bond; and R^{100}, R^{101}, R^{102}, R^{103}, and R^{104} are independently hydrogen, alkyl, alkenyl, alkynyl, hydroxyl, alkoxy, cyano, azido, halogen, nitro, SO_2, SO_3, thioalkyl, or amino.

Subembodiment 9: X is hydrogen, cyano, amino, amido, hydroxyl, 5'-deoxyadenosyl, L-T, alkyl, alkenyl, alkynyl, cylcoalkyl, aryl, aralkyl, heterocycle, or heteroaryl, or alkylheteroaryl; M, K, E and G^1 retain their natural vitamin B_{12} configuration; Y^1, Y^2, Y^3, Y^4, Y^5, Y^6 and Y^7 independently are O, S or NJ^2; V^1, V^2, V^3, V^4, V^5, V^6, V^7 and V^8 independently are O, S or NJ^3; $CR^{102}R^{103}$, or a direct bond; Z^1, Z^2, Z^3, Z^4, Z^5, Z^7 and Z^8 independently are R^{104} or L-T; each L is independently a direct bond or the residue of a multivalent moiety that does not significantly impair the ability of the compound to bind transcobalamin II; each L is independently a direct bond or the residue of a multivalent moiety that does not significantly impair the ability of the compound to bind transcobalamin II; each T independently comprises the residue of one or more molecules comprising B-10 or one or more radionuclides; at least one of Z^2, Z^4, or Z^5 comprises a molecule comprising B-10, the remaining Z moieties retaining their natural vitamin B_{12} configuration; J^1, J^2 and J^3 independently are hydrogen, alkyl, alkenyl, alkynyl, alkaryl, cycloalkyl, aryl, cycloaryl, heterocycle, heteroaryl, hydroxyl, alkoxy, or amine; R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{12}, R^{13}, R^{14} and R^{15} all retain their natural vitamin B_{12} configuration; and R^{100}, R^{101}, R^{102}, R^{103}, and R^{104} are independently hydrogen, alkyl, alkenyl, alkynyl, hydroxyl, alkoxy, cyano, azido, halogen, nitro, SO_2, SO_3, thioalkyl, or amino.

Subembodiment 10: X is 5'-deoxyadenosyl; M, K, E and G^1 retain their natural vitamin B_{12} configuration; Y^1, Y^2, Y^3, Y^4, Y^5, Y^6 and Y^7 independently are O, S or NJ^2; V^1, V^2, V^3, V^4, V^5, V^6, V^7 and V^8 independently are O, S or NJ^3; $CR^{102}R^{103}$, or a direct

bond; Z^1, Z^2, Z^3, Z^4, Z^5, Z^7 and Z^8 independently are R^{104} or L-T; each L is independently a direct bond or the residue of a multivalent moiety that does not significantly impair the ability of the compound to bind transcobalamin II; each L is independently a direct bond or the residue of a multivalent moiety that does not significantly impair the ability of the compound to bind transcobalamin II; each T independently comprises the residue of one or more molecules comprising B-10 or one or more radionuclides; at least one of Z^2, Z^4, or Z^5 comprises a molecule comprising B-10, the remaining Z moieties retaining their natural vitamin B_{12} configuration; J^1, J^2 and J^3 independently are hydrogen, alkyl, alkenyl, alkynyl, alkaryl, cycloalkyl, aryl, cycloaryl, heterocycle, heteroaryl, hydroxyl, alkoxy, or amine; R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{12}, R^{13}, R^{14} and R^{15} all retain their natural vitamin B_{12} configuration; and R^{100}, R^{101}, R^{102}, R^{103}, and R^{104} are independently hydrogen, alkyl, alkenyl, alkynyl, hydroxyl, alkoxy, cyano, azido, halogen, nitro, SO_2, SO_3, thioalkyl, or amino.

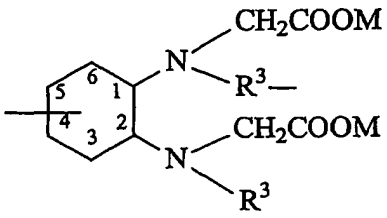
Subembodiments 11-20: Any one of subembodiments 1-10, wherein L is a direct bond and the molecule comprising B-10 comprises an o-carborane, an m-carborane or a p-carborane.

V. Chelating Group

Chelating groups can be used to link radionuclides to the cobalamin conjugate of the present invention. Any suitable chelating group can be employed. Suitable chelating groups include those disclosed in U.S. Patent Number 5,739,313. Other suitable chelating groups are the thiazoline derivatives disclosed in U.S. Patent No. 6,083,966, the pyridinones disclosed in U.S. Patent No. 5,892,029, and the catecholaurates disclosed in U.S. Patent No. 5,514,695.

In one embodiment, the chelating group can be NTA, HEDTA, DCTA, RP414, MDP, DOTATOC, CDTA, HYNIC, EDTA, DTPA, TETA, DOTA, DOTMP, DCTA, 15N4, 9N3, 12N3, or MAG3 (or another suitable polyamino acid chelator), which are described herein below, or a phosphonate chelator (e.g. EDMT). In a preferred embodiment, the chelating group is DTPA.

DTPA is diethylenetriaminepentaacetic acid; TETA is 1,4,8,11-tetraaza-cyclo-tetradecane-N,N',N",N'"-tetraacetic acid; DOTA is 1,4,7,10-tetraaza-cyclododecane-N,N',N",N'"-tetraacetic acid; 15N4 is 1,4,8,12-tetraazacyclo-pentadecane-N,N',N",N'"-tetra-acetic acid; 9N3 is 1,4,7-triazacyclononane-N,N',N"-triacetic acid; 12N3 is 1,5,9-triazacyclo-dodecane-N,N',N"-triacetic acid; polyaminoacid chelators, such as MAG3 is (N-(N-(N-((benzoylthio)acetyl)glycyl)glycyl)glycine); and DCTA is a cyclohexane-based metal chelator of the formula



wherein R^3 may by $(C_1\text{-}C_4)$alkyl or CH_2CO_2-, which may be attached through positions 4 or 5, or through the group R^3 and which carries from 1 to 4 detectable metal or nonmetal cations (M), monovalent cations, or the alkaline earth metals. Thus, with metals of oxidation state +1, each individual cyclohexane-based molecule may carry up to 4 metal cations (where both R^3 groups are CH_2COOM). As is more likely, with higher oxidation states, the number of metals will decrease to 2 or even 1 per cyclohexane skeleton. This formula is not intended to limit the molecule to any specific stereochemistry.

NTA, HEDTA, and DCTA are disclosed in Poster Sessions, Proceedings of the 46th Annual Meeting, J. Nuc. Med., p. 316, No. 1386. RP414 is disclosed in Scientific Papers, Proceedings of the 46th Annual Meeting, J. Nuc. Med., p. 123, No. 499. MDP is disclosed in Scientific Papers, Proceedings of the 46th Annual Meeting, J. Nuc. Med., p. 102, No. 413. DOTATOC is disclosed in Scientific Papers, Proceedings of the 46th Annual Meeting, J. Nuc. Med., p. 102, No. 414 and Scientific Papers, Proceedings of the 46th Annual Meeting, J. Nuc. Med., p. 103, No. 415. CDTA is disclosed in Poster Sessions, Proceedings of the 46th Annual Meeting, J. Nuc. Med., p. 318, No. 1396. HYNIC is disclosed in Poster Sessions, Proceedings of the 46th Annual Meeting, J. Nuc. Med., p. 319, No. 1398.

Bifunctional chelators (i.e., chelating groups) based on macrocyclic ligands in which conjugation is via an activated arm attached to the carbon backbone of the ligand can also be employed as a chelating group, as described by M. Moi *et al.*, J. Amer. Chem.,

Soc., 49, 2639 (1989) (2-p-nitrobenzyl-1,4,7,10-tetraazacyclododecane-N,N',N",N"'-tetraacetic acid); S. V. Deshpande *et al.*, J. Nucl. Med., 31, 473 (1990); G. Kuser *et al.*, Bioconj. Chem., 1, 345 (1990); C. J. Broan *et al.*, J. C. S. Chem. Comm., 23, 1739 (1990); and C. J. Anderson *et al.*, J. Nucl. Med. 36, 850 (1995) (6-bromoacetamido-benzyl-1,4,8,11-tetraazacyclotetadecane-N,N',N",N"'-tetraacetic acid (BAT)).

In addition, the chelator or chelating group can be any of the chelating groups disclosed in Scientific Papers, Proceedings of the 46th Annual Meeting, J. Nuc. Med., Wednesday, June 9, 1999, p. 124, No. 500.

Specifically, the chelating group can be any one of the carbonyl complexes disclosed in Waibel *et al.*, Nature Biotechnology, 897-901, Vol. 17, September 1999; or Sattelberger *et al.*, Nature Biotechnology, 849-850, Vol. 17, September 1999.

Specifically, the detectable chelating group can be any one of the carbonyl complexes disclosed in Waibel *et al.*, Nature Biotechnology, 897-901, Vol. 17, September 1999; or Sattelberger *et al.*, Nature Biotechnology, 849-850, Vol. 17, September 1999, further comprising a metallic radionuclide. More specifically, the detectable chelating group can be any one of the carbonyl complexes disclosed in Waibel *et al.*, Nature Biotechnology, 897-901, Vol. 17, September 1999; or Sattelberger *et al.*, Nature Biotechnology, 849-850, Vol. 17, September 1999, further comprising Technetium-99m, Rhenium-186, or Rhenium-188.

VI. Therapeutic and/or Detectable Radionuclides

In one embodiment the TC- or IF-binding carrier of the present invention is also linked to a detectable radionuclide or other imaging agent. As used herein, a "detectable radionuclide" is any suitable radionuclide (i.e., radioisotope) capable of being detected in a diagnostic procedure *in vivo* or *in vitro*. Suitable detectable radionuclides include metallic radionuclides (i.e., metallic radioisotopes) and non-metallic radionuclides (i.e., non-metallic radioisotopes).

Suitable metallic radionuclides (i.e., metallic radioisotopes or metallic paramagnetic ions) include Antimony-124, Antimony-125, Arsenic-74, Barium-103, Barium-140, Beryllium-7, Bismuth-206, Bismuth-207, Cadmium-109, Cadmium-115m, Calcium-45, Cerium-139, Cerium-141, Cerium-144, Cesium-137, Chromium-51, Cobalt-

55, Cobalt-56, Cobalt-57, Cobalt-58, Cobalt-60, Cobalt-64, Copper-67, Erbium-169, Europium-152, Gallium-64, Gallium-68, Gadolinium-153, Gadolinium-157 Gold-195, Gold-199, Hafnium-175, Hafnium-175-181, Holmium-166, Indium-110, Indium-111, Iridium-192, Iron-55, Iron-59, Krypton-85, Lead-210, Lutetium-177, Manganese-54, Mercury-197, Mercury-203, Molybdenum-99, Neodymium-147, Neptunium-237, Nickel-63, Niobium-95, Osmium-185 + 191, Palladium-103, Platinum-195m, Praseodymium-143, Promethium-147, Protactinium-233, Radium-226, Rhenium-186, Rhenium-188, Rubidium-86, Ruthenium-103, Ruthenium-106, Scandium-44, Scandium-46, Selenium-75, Silver-110m, Silver-111, Sodium-22, Strontium-85, Strontium-89, Strontium-90, Sulfur-35, Tantalum-182, Technetium-99m, Tellurium-125, Tellurium-132, Thallium-204, Thorium-228, Thorium-232, Thallium-170, Tin-113, Tin-114, Tin-117m, Titanium-44, Tungsten-185, Vanadium-48, Vanadium-49, Ytterbium-169, Yttrium-86, Yttrium-88, Yttrium-90, Yttrium-91, Zinc-65, and Zirconium-95.

The compounds of the invention can also comprise one or more (e.g., 1, 2, 3, or 4) non-metallic radionuclide which can be directly linked to a residue of the compound of formula I at any synthetically feasible site, or can be linked to a residue of the compound of formula I, by a linker, at any synthetically feasible site. Suitable linkers are described herein. In addition, suitable points of attachment of the compound of formula I for the non-metallic radionuclide, either directly or by a linker, are also described herein. The invention also provides compounds having more than one non-metallic radionuclide attached to a compound of formula I, either directly, or by a linker.

Specifically, the non-metallic radionuclide can be a non-metallic paramagnetic atom (e.g., Fluorine-19); or non-metallic positron emitting radionuclide (e.g., Carbon-11, Fluorine-18, Iodine-12, or Bromine-76), or a nonmetallic gamma emitting radionuclide such as Iodine-123 or Iodine-131. Fluorine-19 is a suitable non-metallic paramagnetic for use the compounds of the present invention in part because there is typically little or no background noise associated with the diagnostic use of fluorine in the body of a mammal (e.g., human).

As used herein, a "therapeutic chelating group" is a chelating group comprising a metallic radionuclide (e.g., a metallic radioisotope) that possesses therapeutic efficacy against cancer or other neoplastic cells *in vivo* or *in vitro*. Any suitable chelating group can be employed.

Specifically, the therapeutic chelating group can be any of the carbonyl complexes disclosed in Waibel et al., Nature Biotechnology, 897-901, Vol. 17, September 1999; or Sattelberger et al., Nature Biotechnology, 849-850, Vol. 17, September 1999, further comprising a metallic radionuclide. More specifically, the therapeutic chelating group can be any of the carbonyl complexes disclosed in Waibel et al., Nature Biotechnology, 897-901, Vol. 17, September 1999; or Sattelberger et al., Nature Biotechnology, 849-850, Vol. 17, September 1999, further comprising Rhenium-186 or Rhenium-188.

Tumors treatable with the compounds and methods of the invention can be located in any part of the mammal. Specifically, the tumor can be located in the breast, lung, thyroid, lymph node, genitourinary system (e.g., kidney, ureter, bladder, ovary, teste, or prostate), musculoskeletal system (e.g., bones, skeletal muscle, or bone marrow), gastrointestinal tract (e.g., stomach, esophagus, small bowel, colon, rectum, pancreas, liver, or smooth muscle), central or peripheral nervous system (e.g., brain, spinal cord, or nerves), head and neck tumors (e.g., ears, eyes, nasopharynx, oropharynx, or salivary glands), or the heart.

VII. Pharmaceutically Acceptable Salt or Prodrug Formulations

The term "pharmaceutically acceptable salt or prodrug" is used throughout the specification to describe any pharmaceutically acceptable form (such as an ester, mono-, di- or tri-phosphate ester, salt of an ester or a related group) of a TC- or IF- binding carrier, which, upon administration to a patient, provides the active compound. Pharmaceutically acceptable salts include those derived from pharmaceutically acceptable inorganic or organic bases and acids. Suitable salts include those derived from alkali metals such as potassium and sodium, alkaline earth metals such as calcium and magnesium, among numerous other acids well known in the pharmaceutical art. Pharmaceutically acceptable prodrugs refer to a compound that is metabolized, for example hydrolyzed or oxidized, in the host to form the compound of the present invention. Typical examples of prodrugs include compounds that have biologically labile protecting groups on a functional moiety of the active compound. Prodrugs include compounds that can be oxidized, reduced, aminated, deaminated, hydroxylated, dehydroxylated, hydrolyzed, dehydrolyzed, alkylated, dealkylated, acylated, deacylated,

phosphorylated, dephosphorylated to produce the active compound. The compounds of this invention possess activity against infectious disease or are metabolized to a compound that exhibits such activity.

In cases where compounds are sufficiently basic or acidic to form stable nontoxic acid or base salts, administration of the compound as a pharmaceutically acceptable salt may be appropriate. Examples of pharmaceutically acceptable salts are organic acid addition salts formed with acids, which form a physiological acceptable anion, for example, tosylate, methanesulfonate, acetate, citrate, malonate, tartarate, succinate, benzoate, ascorbate, α-ketoglutarate and α-glycerophosphate. Suitable inorganic salts may also be formed, including, sulfate, nitrate, bicarbonate and carbonate salts.

Pharmaceutically acceptable salts may be obtained using standard procedures well known in the art, for example by reacting a sufficiently basic compound such as an amine with a suitable acid affording a physiologically acceptable anion. Alkali metal (for example, sodium, potassium or lithium) or alkaline earth metal (for example calcium) salts of carboxylic acids can also be made.

Any of the TC- or IF-binding carriers described herein can be administered as a prodrug to increase the activity, bioavailability, stability or otherwise alter the properties of the carrier. A number of prodrug ligands are known. In general, alkylation, acylation or other lipophilic modification of the mono, di or triphosphate of the G^1 substituent on the five-membered "sugar-ring" moiety will increase the stability of the carrier. Examples of substituent groups that can replace one or more hydrogens on the phosphate moiety are alkyl, aryl, steroids, carbohydrates, including sugars, 1,2-diacylglycerol and alcohols. Many are described in R. Jones and N. Bischofberger, *Antiviral Research*, 27 (1995) 1-17. Any of these can be used in combination with the disclosed carriers to achieve a desired effect.

The G^1 substituent of the active carrier can also be provided as a 5'-phosphoether lipid or a 5'-ether lipid, as disclosed in the following references, which are incorporated by reference herein: Kucera, L.S., N. Iyer, E. Leake, A. Raben, Modest E. K., D. L. W., and C. Piantadosi. 1990. "Novel membrane-interactive ether lipid analogs that inhibit infectious HIV-1 production and induce defective virus formation." *AIDS Res. Hum. Retro Viruses*. 6:491-501; Piantadosi, C., J. Marasco C. J., S. L. Morris-Natschke, K. L. Meyer, F. Gumus, J. R. Surles, K. S. Ishaq, L. S. Kucera, N. Iyer, C. A. Wallen, S.

Piantadosi, and E. J. Modest. 1991. "Synthesis and evaluation of novel ether lipid nucleoside conjugates for anti-HIV activity." *J. Med. Chem.* **34**:1408.1414; Hosteller, K. Y., D. D. Richman, D. A. Carson, L. M. Stuhmiller, G. M. T. van Wijk, and H. van den Bosch. 1992. "Greatly enhanced inhibition of human immunodeficiency virus type 1 replication in CEM and HT4-6C cells by 3'-deoxythymidine diphosphate dimyristoylglycerol, a lipid prodrug of 3,-deoxythymidine." *Antimicrob. Agents Chemother.* **36**:2025.2029; Hosetler, K. Y., L. M. Stuhmiller, H. B. Lenting, H. van den Bosch, and D. D. Richman, 1990. "Synthesis and antiretroviral activity of phospholipid analogs of azidothymidine and other antiviral nucleosides." *J. Biol. Chem.* 265:61127.

Nonlimiting examples of U.S. patents that disclose suitable lipophilic substituents that can be covalently incorporated into the TC- or IF-binding agent, preferably at the G^1 position of the carrier or lipophilic preparations, include U.S. Patent Nos. 5,149,794 (Sep. 22, 1992, Yatvin *et al.*); 5,194,654 (Mar. 16, 1993, Hostetler *et al.*, 5,223,263 (June 29, 1993, Hostetler *et al.*); 5,256,641 (Oct. 26, 1993, Yatvin *et al.*); 5,411,947 (May 2, 1995, Hostetler *et al.*); 5,463,092 (Oct. 31, 1995, Hostetler *et al.*); 5,543,389 (Aug. 6, 1996, Yatvin *et al.*); 5,543,390 (Aug. 6, 1996, Yatvin *et al.*); 5,543,391 (Aug. 6, 1996, Yatvin *et al.*); and 5,554,728 (Sep. 10, 1996; Basava *et al.*), all of which are incorporated herein by reference. Foreign patent applications that disclose lipophilic substituents that can be attached to the carrier of the present invention, or lipophilic preparations, include WO 89/02733, W0 90/00555, W0 91/16920, W0 91/18914, W0 93/00910, W0 94/26273, W0 96/15132, EP 0 350 287, EP 93917054.4, and W0 91/19721.

VIII. Stereoisomerism and Polymorphism

Compounds of the present invention having a chiral center may exist in and be isolated in optically active and racemic forms. Some compounds may exhibit polymorphism. The present invention encompasses racemic, optically-active, polymorphic, or stereoisomeric form, or mixtures thereof, of a compound of the invention, which possess the useful properties described herein. The optically active forms can be prepared by, for example, resolution of the racemic form by recrystallization techniques, by synthesis from optically-active starting materials, by chiral synthesis, or by chromatographic separation using a chiral stationary phase or by enzymatic resolution.

IX. Transport Proteins

In humans, the average daily intake (in a Western diet) of vitamin B_{12} is about 4-5 g. Additional synthesis of cobalamin may be produced in the ileum and the right colon, but in an unknown amount. The total lumenal cobalamin that must be assimilated each day in humans is estimated at 7-14 g, the sum of the dietary and endogenous cobalamin. Intestinal epithelial cells possess carriers and transporters that are highly efficient in the uptake of the small products of digestion, such as vitamins, minerals and amino acids. These mechanisms are necessary for the uptake of these molecules, as the epithelial cell layer presents an almost impenetrable barrier to peptides larger than five or six amino acids in size. The cobalamins of the present invention are large molecules that are not absorbed directly from the intestine, as they are too big to diffuse across the intestinal wall. Therefore, the absorption of the cobalamins is dependent upon transport proteins. The uptake of vitamin B12 from the intestine to the blood is perhaps the most complex uptake mechanism of all the vitamins, involving at least four separate cobalamin binding proteins and receptors.

Three distinct groups of transport proteins are involved in the absorption and transport of cobalamins: intrinsic factor (IF), haptocorrin (HC; also called R-protein; in which transcobalamin I (TC-I) and transcobalamin III (TC-III) are members) and transcobalamin II (TC-II). Both IF and TC II deficiencies lead to abnormalities such as megaloblastic anemia and demyelinating disorder of the nervous system. Each protein only has one subunit and one binding site to cobalamin. IF is a 45 kDa (in humans) to 55 kDa (in hogs) plasma glycoprotein with 15% carbohydrate content. HC's are 58 kDa (in humans) to 60 kDa (in rabbits) plasma glycoproteins of 33-40% carbohydrate content with 16-19 sialic acid residues. Human TC-II is a 43 kDa plasma protein (in humans) with 0% carbohydrate content. Each binding protein has a separate affinity for cobalamin, as well as separate cell receptors. Generally, cobalamin is initially bound by HC in the stomach, followed by IF in the small intestine. An IF receptor is then involved in the uptake of the IF-cobalamin complex by the intestinal epithelial cell, leading to the proteolytic release of cobalamin, and subsequent binding to TC-II.

Intrinsic factor (IF) and haptocorrin (HC; are the main intestinal lumenal cobalamin binders. In particular IF is of particular relevance to the field of oral peptide and protein delivery. Therefore, IF is mainly produced in the gastric body and medium

sized ducts and HC is mainly produced in granulocytes, the yolk sac, mammary glands, salivary acini and ducts. In general, in plasma or serum, cobalamin is also bound to HC (derived from white cells) or to TC-II. The former complex is taken up by the liver, delivering free cobalamin to the intestinal lumen as the first limb of an enterohepatic circulation.

IF is the most specific of the cobalamin-binding proteins. Cyanocobalamin, hydoxy-cobalamin (HOCbl), methylcobalamin (MeCbl) and adenyosylcobalamin (AdoCbl) bind to intrinsic factor with similar affinities, thereby suggesting that the upper - axial ligand of the cobalt does not influence the binding significantly. However, after dietary release of vitamin B_{12}, the affinity for the cobalamin for IF is reduced, due to the low pH. Rather, the released vitamin B_{12} is preferentially bound to salivary HC, as HC may protect the vitamin from acid hydrolysis (possibly due to the extensive glycosylation of HC).

HC comprises a group of immunologically identical proteins secreted into many body fluids (plasma, milk, amniotic fluid, saliva and gastric juices) from many types of cells (granulocytes, mammary glands, yolk sac or visceral placental membranes, salivary duct and acinar cells, and gastric mucosa of some species). These proteins were known previously as R proteins (for rapid electrophoresis), non-intrinsic factors or transcobalamin I and III. They are characterized by different glycosylation processes and account for much of the total bound cobalamin in the serum (about 80% of bound cobalamin in serum). HC turns over very slowly ($t_{1/2}$ = 10 days) and appears to serve as the major storage protein for cobalamin and may also stabilize serum cobalamin against transdermal photolysis (Allen, R. H. Prog Hematol. **1975**, *9*, 57-84).

Within the proximal small intestine, HC is degraded by pancreatic enzymes, freeing cobalamin to combine with other transport proteins, most notably IF. In contrast to HC's, the IF-cobalamin complex is resistant to proteolytic digestion. Once the cobalamin-transport protein is internalized via receptor-mediated endocytosis, the cobalamin is cleaved from transport protein via protease(s) and bound to transcobalamin II (TC II). From there, the TC II-cobalamin complex is used for the transport of absorbed cobalamin to peripheral tissues. Therefore, TC-II is found in most tissues. Antibodies to TC II inhibit the transport of cobalamins and block the proliferation of leukemic cells in vitro (McLean, G. R. et al. Blood, **1997** , *89*, 235-242). In cow's milk, in particular, the

major cobalamin binder is not HC, but rather TC-II (Fedosov, S. N. et al. Biochemistry **1995**, *34*, 16082-16087 and Fedosov, S. N. et al.Biochim. Biophys. Acta. **1996**, *1292*, 113-119).

Early attempts to purify transport proteins utilized conventional techniques such as ammonium sulfate fractionation, ion exchange and size exclusion chromatography. These methods yielded a product that was devoid of the other types transport proteins, and in particular, separation of TC-II from TC-I was possible, but contained other plasma proteins. The introduction of affinity chromatography provided pure proteins even in extremely low concentrations. Three main types of affinity columns have been used to purify the transport proteins, in particular, columns containing cobalamin coupled to different matrices. The first was a monocarboxylic acid derivative of cobalamin linked to Sepharose beads via a diamino-dipropylamine spacer arm (Allen, R. H. et al. J. Biol Chem. **1972**, *247*, 7695-7701 and Allen, R. H. et al. J. Biol Chem. **1973**, *248*, 3660-3669). However, it may be necessary to use a chaotropic reagent to elute the protein from the matrix, possibly resulting in a denatured transport protein, which may not be able to renature. For instance, the elution of the bound protein from Cohn fraction III of human plasma, a mixture that contains 27-40% of the plasma TC-II, required the use of guanidine hydrochloride to release the denatured TC-II, which could not be renatured.

To avoid the use of chaotropic reagents, temperature- or photolabile linkages between the cobalamin and the insoluble matrix were developed (Nexo, E. "Cobalamin binding proteins," in *Vitamin B_{12} and B_{12}-proteins*, eds Krantler, B.; Arigoni, D. and Golding, B. T.; Wiley & Sons, Ltd. 461-475). Matrices formed in this manner are able to release the transport protein by dissociating the cobalamin from the matrix, thus providing the transport protein saturated with cobalamin, circumventing the denaturant effect of chaotrophic agents.

In a preferred embodiment, for large scale purification of transport protein, ion exchange chromatography or ammonium sulfate fractionation is used prior to the purification of the transport protein via an affinity column to concentrate the sample. In an alternate embodiment, ion exchange or size exclusion chromatography is used subsequent to the purification of the transport protein via an affinity column.

X. Synthetic Techniques

Figure 2 and **Figure 3** each illustrate the four step synthesis used to prepare three cyanocobalamin-nido-carborane conjugates. o-Carborane carboxylic acid (2) was prepared by reacting o-carborane with n-butyllithium and carbon dioxide in ether for approximately one hour at -78°C. Treatment with thionyl chloride gave o-carborane carboxylic acid chloride (3), which was allowed to react with 1,4-butanediamine in pyridine to give the amide linked nido-carboranoyl(4-aminobutyl)amide (4). Compound (4) was linked to the b-monocarboxylic acid of cyanocobalamin, the d-monocarboxylic acid of cyanocobalamin or both the b- and d-dicarboxylic acid of cyanocobalamin. In each reaction, hydroxybenzotriazole and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide was added to facilitate the formation of the amide bond.

Other synthetic techniques are generally known. For example, compounds wherein the residue of a molecule comprising B-10 is linked to the 6-position of a compound of formula I can be prepared by reducing a corresponding Co (III) compound of formula I to form a nucleophilic Co (II) compound and treating this Co (II) compound with a residue of a molecule comprising B-10 (or a derivative thereof) comprising a suitable leaving group, such as a halide (e.g., a chloride). Compounds wherein the linker is linked to the 6-position of a compound of formula I can be prepared by preparing a nucleophilic Co (II) species as described herein above, and reacting it with a linker comprising a suitable leaving group, such as a halide (e.g. a chloride). Peptides and amino acids can be attached to the 6-position by reducing a corresponding Co (III) compound of formula I to form a nucleophilic Co (II) compound, and treating the Co (II) compound with a suitable alkylating agent comprising an amino acid or peptide.

Coupling of L-T to the ribose moiety at K or G^1 may be accomplished by activating the natural OH at either K or G^1 with a suitable reagent such as succinic anhydride, to yield a reactive group such as a carboxylate. This technique is described in detail in Toraya, Bioinorg. Chem. 4:245-255, 1975.

Coupling of L-T to M can be accomplished using techniques described in detail in Jacobsen, Anal. Biochem. 113:164-171, 1981.

The residue of vitamin B_{12} or its analog can be prepared by any suitable means known in the art. For example, a monocarboxylic acid or dicarboxylic acid of cobalamin

can be prepared as disclosed in U.S. patent No. 5,739,313. These compounds can be prepared by the mild acid hydrolysis of cyanocobalamin, which has been shown to yield a mixture of mono-, a dicarboxylic acid and one tricarboxylic acid. These carboxylic acids are derived from the propionamide side chains designated b, *d- and e-*, as discussed hereinabove, which are more susceptible to hydrolysis than the amide groups on acetamide side chains a-, c-, and g-. The b-, d-, and e-monocarboxylic acids can be separated by column chromatography. L. Anton *et al.*, J. Amer. Chem. Soc.,102, 2215 (1980). See, also, J B. Armitage *et al.*, L Chem. Sot., 3349 (1953); K. Bernhauer, Biochem. Z., 344, 289 (1966); H. P. C. Hogenkamp *et al.*, Biochemistry, 14, 3707 (1975); and L. Ellenbogen, in "Cobalamin," Biochem. and Pathophysiol, B. Babior, ed., Wiley, N.Y. (1975) at chapter 5.

Additional compounds, intermediates, and synthetic preparations thereof are disclosed, for example, in Hogenkamp, H. *et al., Synthesis and Characterization of nido-Carborane-Cobalamin Conjugates*, Nucl. Med. & Biol., 2000, 27, 89-92; Collins, D., *et al., Tumor Imaging Via Indium 111-Labeled DTPA-Adenosylcobalamin*, Mayo Clinic Proc., 1999, 74:687-691.

XI. Examples of Disorders Characterized by Abnormal Cellular Proliferation

Non-limiting examples of proliferative disorders other than neoplasms that can be treated with a TC- or IF- binding carrier linked to a neutron capture therapy agent include those in **Table 1**, as well as any others listed or described in the Background of the Invention or otherwise in the specification.

Table 1

Organ System	Disease/Pathology
Dermatological	Psoriasis (all forms), acne vulgaris, acne rosacea, common warts, anogenital (venereal) warts, eczema; lupus associated skin lesions; dermatitides such as seborrheic dermatitis and solar dermatitis; keratoses such as seborrheic keratosis, senile keratosis, actinic keratosis, photo-induced keratosis, skin aging, including photo-induced skin aging, keratosis follicularis, keloids and Prophylaxis against keloid formation; leukoplakia, lichen, planus, keratitis, contact dermatitis, eczema, urticaria, pruritus, hidradenitis, acne inversa
Cardiovascular	Hypertension, vasculo-occlusive diseases including Atherosclerosis, thrombosis and restenosis after angioplasty; acute coronary syndromes such as unstable angina, myocardial infarction, ischemic and non-ischemic cardiomyopathies, post-MI cardiomyopathy and myocardial fibrosis, substance-induced cardiomyopathy.
Endocrine	Insulin resistant states including obesity, diabetes mellitus (types 1 & 2), diabetic retinopathy, macular degeneration associated with diabetes, gestational diabetes, impaired glucose tolerance, polycystic ovarian syndrome; osteoporosis, osteopenia, accelerated aging of tissues and organs including Werner's syndrome.
Urogenital	Endometriosis, benign prostatic hyperplasia, leiomyoma, Polycystic kidney disease, diabetic nephropathy.
Pulmonary	Asthma, chronic obstructive pulmonary disease (COPD), reactive Airway disease, pulmonary fibrosis, pulmonary hypertension.

Organ System	Disease/Pathology
Connective tissue/joints	Immunological Rheumatoid arthritis, Raynaud's phenomenon/disease, Sjogren's Syndrome systemic sclerosis, systemic lupus erythematosus, vasculitides, ankylosing spondylitis, osteoarthritis, reactive arthritis, psoriatic arthritis, fibromyalgia.
Other	Fibrocystic breast disease, fibroadenoma, chronic fatigue syndrome.

Nonlimiting examples of neoplastic diseases or malignancies treatable with the TC- or IF-binding carrier linked to a neutron capture agent are listed in **Table 2.**

Table 2

Organ System	Malignancy/Cancer type
Skin	Basal cell carcinoma, melanoma, squamous cell carcinoma; cutaneous T cell lymphoma; Kaposi's sarcoma.
Hematological	Acute leukemia, chronic leukemia and myelodysplastic syndromes.
Urogenital	Prostatic, renal and bladder carcinomas, anogenital carcinomas including cervical, ovarian, uterine, vulvar, vaginal, and those associated with human papilloma virus infection.
Neurological	Gliomas including glioblastomas, astrocytoma, ependymoma, medulloblastoma, oligodendroma; meningioma, pituitary adenoma, neuroblastoma, craniopharyngioma.
Gastrointestinal	Colon, colorectal, gastric, esophageal, mucocutaneous carcinomas.
Breast	Breast cancer including estrogen receptor and progesterone Receptor positive or negative subtypes, soft tissue tumors.
Metastasis	Metastases resulting from the neoplasms.

Organ System	Malignancy/Cancer type
Skeletal	Osteogenic sarcoma, malignant fibrou histeocytoma, chondrosarcoma, rhabdomyosarcoma, leiomyosarcoma, myeloma.
Diffuse Tumors	Lymphoma (non-Hodgkin's or Hodgkin's), sickle cell anemia.
Other	Angiomata, angiogenesis associated with the neoplasms.

XII. Pharmaceutical Compositions and Administration

Preferred modes of administration of the TC- or IF-binding carriers and neutron capture agents are parenteral, intravenous, intradermal, intra-articular, intra-synovial, intrathecal, intra-arterial, intracardiac, intramuscular, subcutaneous, intraorbital, intracapsular, intraspinal, intrasternal, topical, transdermal patch, via rectal, vaginal or urethral suppository, peritoneal, percutaneous, nasal spray, surgical implant, internal surgical paint, infusion pump, or via catheter. In one embodiment, the agent and carrier are administered in a slow release formulation such as an implant, bolus, microparticle, microsphere, nanoparticle or nanosphere. For standard information on pharmaceutical formulations, see Ansel, et al., *Pharmaceutical Dosage Forms and Drug Delivery Systems*, Sixth Edition, Williams & Wilkins (1995).

The TC- or IF-binding carriers/neutron capture agents can, for example, be administered intravenously or intraperitoneally by infusion or injection. Solutions of the substance can be prepared in water, optionally mixed with a nontoxic surfactant. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, triacetin and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The pharmaceutical dosage forms suitable for injection or infusion can include sterile aqueous solutions or dispersions or sterile powders comprising the substance which are adapted for the extemporaneous preparation of sterile injectable or infusible solutions or dispersions, optionally encapsulated in liposomes. In all cases, the ultimate dosage form must be sterile, fluid and stable under the conditions of manufacture and storage.

The liquid carrier or vehicle can be a solvent or liquid dispersion medium comprising, for example, water, normal saline, ethanol, a polyol (for example, glycerol, propylene glycol, liquid polyethylene glycols, and the like), vegetable oils, nontoxic glyceryl esters, and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the formation of liposomes, by the maintenance of the required particle size in the case of dispersions or by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, benzyl alcohol, sorbic acid, thimerosal and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, buffers or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the substance in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filter sterilization. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze drying techniques, which yield a powder of the active ingredient plus any additional desired ingredient present in the previously sterile-filtered solutions.

Injectable solutions are particularly advantageous for local administration of the therapeutic composition. In particular, parenchymal injection can be used to deliver the therapeutic composition directly to a tumorous growth. Intra-articular injection is a preferred alternative in cases of arthritis where the practitioner wishes to treat one or only a few (such as 2-6) joints. Additionally, the therapeutic compounds are injected directly into lesions (intra-lesion administration) in appropriate cases. Intradermal administration is an alternative for dermal lesions.

The therapeutic compound is optionally administered topically by the use of a transdermal therapeutic system (see, Barry, Dermatological Formulations, (1983) p. 181 and literature cited therein). Transdermal drug delivery (TDD) has several advantages over oral delivery. When compared to oral delivery, TDD avoids gastrointestinal drug metabolism, reduces first pass effects and provides a sustained release of drugs for up to seven days (Elias, et al. Percutaneous Absorption: Mechanisms-Methodology-Drug

Delivery; Marcel Dekker, NY: 1, 1989). This method is especially useful with many therapeutic proteins that are susceptible to gastrointestinal degradation and exhibit poor gastrointestinal uptake. When compared to injections, TDD eliminates the associate pain and the possibility of infection. While such topical delivery systems have been designed largely for transdermal administration of low molecular weight drugs, by definition they are capable of percutaneous delivery. They can be readily adapted to administration of the therapeutic compounds of the invention by appropriate selection of the rate-controlling microporous membrane. Topical application can also be achieved by applying the compound of interest, in a cream, lotion, ointment, or oil based carrier, directly to the skin. Typically, the concentration of therapeutic compound in a cream, lotion or oil is 1-2%.

For drug targeting to lung tissue, the therapeutic compound is formulated into a solution, suspension, aerosol or particulate dispersion appropriate for application to the pulmonary system. The therapeutic agent may be inhaled via nebulizer, inhalation capsule, inhalation aerosol, nasal solution, intratracheal as a solution via syringe, or endotracheal tube as an aerosol or via as a nebulizer solution. Aerosols are prepared using an aqueous aerosol, liposomal preparation or solid particles containing the compound. A nonaqueous (e.g. fluorocarbon propellant) suspension could be used. Sonic nebulizers are preferred because they minimize exposing the therapeutic compound to shear, which can result in degradation of the compound.

Delivery of the cobalamin conjugates of the instant invention by the mucosal route also offers an attractive administration alternative. The prototype formulation for nasal solutions will contain the vitamin B_{12} conjugate dissolved in a suitable aqueous or non-aqueous solvent such as propylene glycol, an antioxidant and aromatic oils as flavoring agents. The formulation may also contain suitable propellant(s).

For ophthalmic applications, the therapeutic compound is formulated into solutions, suspensions and ointments appropriate for use in the eye. For opthalmic formulations, see Mitra (ed.), Ophthalmic Drug Delivery Systems, Marcel Dekker, Inc., New York, New York (1993), and also Havener, W. H., Ocular Pharmacology, C.V. Mosby Co., St. Louis (1983).

Useful dosages of the compounds of formula I can be determined by comparing their *in vitro* activity, and *in vivo* activity in animal models. Methods for the extrapolation of effective dosages in mice, and other animals, to humans are known to the art; for

example, see U.S. Patent No. 4,938,949. The amount of the substance required for use in treatment will vary not only with the particular salt selected but also with the route of administration, the nature of the condition being treated and the age and condition of the patient and will be ultimately at the discretion of the attendant physician or clinician.

In general, however, a suitable dose for nuclear medicine (using a radioactive imaging agent) will be in the range of from about 0.1 μg/patient to about 1000 μg/patient, from about 0.5 to about 500 μg/patient, or from 1 μg/patient to about 100 μg/patient.

A suitable dose for imaging medicine (using a paramagnetic imaging agent) will be in the range of from about 0.1 mg/patient to about 100 mg/patient, from about 0.5 to about 50 mg/patient, or from 1 mg/patient to about 10 mg/patient.

For therapeutic applications, a suitable dose will be in the range of from about 0.05 picograms/kilogram to about 100 mg/kg, from about 10 to about 75 mg/kg of body weight per day, such as 3 to about 50 mg per kilogram body weight of the recipient per day, preferably in the range of 6 to 90 mg/kg/day, most preferably in the range of 15 to 60 mg/kg/day. The substance is conveniently administered in unit dosage form; for example, containing 5 to 1000 mg, conveniently 10 to 750 mg, most conveniently, 50 to 500 mg of active ingredient per unit dosage form.

Ideally, the substance should be administered to achieve peak plasma concentrations of from about 0.05 to about 100 μM, preferably, about 1 to 50 μM, most preferably, about 2 to about 30 μM. This may be achieved, for example, by the intravenous injection of a 0.005 to 10% solution of the substance, optionally in saline, or orally administered as a bolus containing about 0.5-250 mg of the substance. Desirable blood levels may be maintained by continuous infusion to provide about 0.01-5.0 mg/kg/hr or by intermittent infusions containing about 0.4-15 mg/kg of the substance.

The substance may conveniently be presented in a single dose or as divided doses administered at appropriate intervals, for example, as two, three, four or more sub-doses per day.

The cobalamin conjugates may be administered orally in combination with a pharmaceutically acceptable vehicle such as an inert diluent or an edible carrier. They may be enclosed in hard or soft shell gelatin capsules, may be compressed into tablets, or may be incorporated directly with the food of the patient's diet. For oral therapeutic

administration, the substance may be combined with one or more excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at least 0.1% of the substance. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 2 to about 60% of the weight of a given unit dosage form. The amount of substance in such therapeutically useful compositions is such that an effective dosage level will be obtained.

Tablets, troches, pills, capsules, and the like may also contain the following: binders such as gum tragacanth, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, fructose, lactose or aspartame or a flavoring agent such as peppermint, oil of wintergreen, or cherry flavoring may be added. When the unit dosage form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier, such as a vegetable oil or a polyethylene glycol. Various other materials may be present as coatings or to otherwise modify the physical form of the solid unit dosage form. For instance, tablets, pills, or capsules may be coated with gelatin, wax, shellac or sugar and the like. A syrup or elixir may contain the active compound, sucrose or fructose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavoring such as cherry or orange flavor. Of course, any material used in preparing any unit dosage form should be pharmaceutically acceptable and substantially non-toxic in the amounts employed. In addition, the substance may be incorporated into sustained-release preparations and devices.

Sublingual tablets are designed to dissolve very rapidly. Examples of such formulations include ergotamine tartrate, isosorbide dinitrate, isoproterenol HCl. The formulation of these tablets contain, in addition to the drug, a limited number of soluble excipients, usually lactose and powdered sucrose, but occasionally dextrose and mannitol. The process of making sublingual tablets involves moistening the blended powder components with an alcohol-water solvent system containing approximately 60% alcohol and 40% water.

In addition to the cobalamin conjugate, the prototype formulation for sublingual tablets may contain a binder such as povidone or HPMC, diluents such as lactose,

mannitol, starch or cellulose, a disintegrant such as pregelatinized or modified starch, lubricants such as magnesium stearate, stearic acid or hydrogenated vegetable oil, a sweetener such as saccharin or sucrose and suitable flavoring and coloring agents.

It is preferred that the TC binding carrier and the neutron capture agent be administered parenterally, not orally, to increase bioavailability and delivery to proliferative tissue. Importantly, it has been discovered that oral administration of the TC- or IF-binding carrier/neutron capture agent provides insufficient bioavailability to treat proliferative disorders. It is important, and perhaps essential, to administer the neutron capture agent in a manner that does not rely on the ileal intrinsic factor receptor binding absorption pathway of the active agent.

The TC- or IF-binding carrier and the neutron capture agent can be administered in the course of surgical or medical treatment of the afflicted site. For example, the TC- or IF-binding carrier and neutron capture agent can be positioned directly at the site of proliferation during the course of surgery either by painting the formulation onto the surface of the afflicted area (with or without a carrier matrix) or by depositing a bolus of material in a suitable matrix that is released into the afflicted area over time. In another embodiment, the TC- or IF-binding carrier and the neutron capture agent are administered directly into the proliferative mass via injection or catheter.

In the case of nonmalignant or malignant tumor treatment, the TC-binding or IF-binding carrier and neutron capture agent can be effectively administered and neutron capture therapy initiated prior to surgical resection to shrink the tumor and thus decrease the possibility of the uncontrollable spread of diseased cells during the resection.

In one embodiment, the agent and carrier are administered in a slow release formulation such as an implant, bolus, microparticle, microsphere, nanoparticle or nanosphere. Nonlimiting examples of sustained release compositions include semipermeable polymer matrices in the form of shaped articles, e.g., films, microcapsules or microspheres. Sustained release matrices include, for example, polylactides (U.S. Patent No. 3,773,919), copolymers of L-glutamic acid and γ-ethyl-L-glutamate (Sidman *et al.*, Biopolymers 22:547-556, 1983), or poly-D-(-)-3-hydroxybutyric acid (EP 133,988). Sustained release compositions also include one or more liposomally entrapped compounds of formula I. Such compositions are prepared by methods known per se, e.g., as taught by Epstein *et al.* Proc. Natl. Acad. Sci. USA 82:3688-3692, 1985. Ordinarily,

the liposomes are of the small (200-800 Å) unilamellar type in which the lipid content is greater than about 30 mol % cholesterol, the selected proportion being adjusted for the optimal therapy.

A number of sustained-release implants are known in the art. Most implants are "matrix" type, and comprise an active compound dispersed in a matrix of a carrier material. The carrier material may be either porous or non-porous, solid or semi-solid, and permeable or impermeable to the active compound. Matrix devices are typically biodegradable, i.e., they slowly erode after administration. Alternatively, matrix devices may be nondegradable, and rely on diffusion of the active compound through the walls or pores of the matrix. Matrix devices are preferred for the applications contemplated herein.

Thus, in one embodiment the invention provides a surgical implant for localized delivery of an anti-proliferative agent comprising the cobalamin conjugate of the present invention, and a biodegradable binder. The implant preferably is capable of releasing and delivering the cobalamin conjugate to substantially all of an area of clear margin that results from a surgical resection, and is also preferably capable of releasing the cobalamin conjugate at a substantially constant rate. In another embodiment the invention provides a method of delivering a neutron capture agent to an area of clear margin following a surgical resection comprising (i) providing an implant comprising a TC- or IF-binding carrier linked to a neutron capture agent and a biodegradable binder; and (ii) placing the implant into a void created by surgical resection.

The surgical implant can exhibit a variety of forms. In one embodiment the implant is a bolus, comprising a viscous and deformable material capable of being shaped and sized before or during implantation to complement a void created by a surgical resection, and sufficiently deformable upon implantation to contact substantially all of an area of clear margin. The surgical implant can also comprising a plurality of capsules that can be poured into the void created by a surgical resection. These capsules will contain the cobalamin conjugate and a suitable binder. Because they are flowable, they can be poured into the void created by a surgical lumpectomy, and thereby contact substantially all of the areas of clear margin.

Many suitable compositions for the implant are known and can be used in practicing the invention. Such compositions are described in, for example, Chasin et al., Biodegradable Polymers as Drug Delivery Systems, Marcel Dekker Inc., NY, ISBN 0-

8247-8344-1. Preferable compositions are pharmaceutically acceptable, biodegradable, and meet the particular release profile characteristics that are required to achieve the administration regime involved.

The implant typically comprises a base composition that acts as a matrix to contain and hold the contents of the implant together. The base composition can, in turn, comprise one or more constituents. Examples of base compositions include polymers and copolymers of anhydrides, orthoester, lactic acid, glycolic acid, dioxonane, trimethylene carbonate, ε-caprolactone, phosphazene and glyceryl monostearate.

In one embodiment the base composition for the matrix comprises a polyanhydride, which can be synthesized via the dehydration of diacid molecules by melt condensation. Degradation times can be adjusted from days to years according to the hydrophobicity of the monomer selected. The materials degrade primarily by surface erosion and possess excellent in vivo compatibility. In one embodiment the polyanhydride is formed from sebasic acid and hexadecandioic acid (poly(SA-HDA anhydride). Wafer-like implants using this base composition have been approved for use in brain cancer, as Giadel®, by Guilford Pharmaceuticals.

The implant optionally can comprise erosion and biodegradation enhancers that facilitate the erosion of the matrix, the dissolution of the core composition, or the uptake of the core composition via metabolic processes. Particularly suitable erosion and biodegradation enhancers are biodegradable in biological fluids, and biocompatible. Hydrophilic constituents are typical, because they are capable of enhancing the erosion of the implant in the presence of biological fluids. For example, K. Juni *et al.*, Chem. Pharm. Bull., 33, 1609 (1985) disclose that the release rate of bleomycin from polylactic acid microspheres is greatly enhanced by incorporating fatty acid esters into the microspheres. Other exemplary hydrophilic constituents are described, for example, in Wade & Weller, Handbook of pharmaceutical Excipients (London: Pharmaceutical Press; Washington D.C.: American Pharmaceutical Ass'n 1995), and include the polyethylene glycols ("PEGs"), propylene glycol ("PG"), glycerin, and sorbitol.

Surfactants further enhance the erosion of the matrix and the release of the drug. Surfactants are generally capable of increasing the wettability and the solubility of the base composition in biological fluids, and thereby causing the disintegration and erosion of the implant. Surfactants can also help to break down the core composition matrix

when, for example, the method of forming the dosage form has reduced the solubility of any of the constituents. Surfactants can also improve the uptake of the dosage forms into the bloodstream. Suitable surfactants include, for example, glyceryl based surfactants such as glyceryl monooleate and glyceryl monolaurate, poloxamers such as Pluronic F127, and polysorbates such as polyoxyethylene sorbitan monooleate ("Tween 80").

The implant could also include components that retard the rate at which the implant erodes or biodegrades (erosion and/or biodegradation retardants). Hydrophobic constituents are a particularly suitable class of components for retarding the rate at which the outer layer biodegrades. Suitable hydrophobic constituents are described, for example, in the Handbook of Pharmaceutical Excipients, the disclosure from which being hereby incorporated by reference. Exemplary hydrophobic constituents include peanut oil, olive oil and castor oil.

Any proportions or types of constituents can be chosen that effectively achieve a desired release profile, and thereby carry out the prescribed administration regime. The most desirable base compositions generally release the drug substantially continuously, and biodegrade completely shortly after substantially all of the drug has been effectively released. The amount of drug included in the dosage forms is determined by the total amount of the drug to be administered, and the rate at which the drug is to be delivered. The total amount of the drug to be delivered is determined according to clinical requirements, and in keeping with the considerations that typically inform drug dosage determinations in other contexts. The surgical implant also can contain one or more other drugs having therapeutic efficacy in the intended applications, such as an antibiotic, an analgesic or an anesthetic.

XIII. Controlled Release Formulations

The TC- or IF-binding carrier and neutron capture agent is optionally administered in a controlled release formulation, which can be a degradable or nondegradable polymer, hydrogel, organogel, or other physical construct that modifies the bioabsorption, half life or biodegradation of the TC- or IF-binding carrier/neutron capture agent. The controlled release formulation can be a material that is painted or otherwise applied onto the afflicted site, either internally or externally. In one embodiment, the invention provides a

biodegradable bolus or implant that is inserted into the pocket created by surgical resection of a tumor, or directly into the tumor itself. In another example, the controlled release formulation can be applied to a psoriatic lesion, eczema, atopic dermatitis, lichen planus, wart, pemphigus vulgaris, actinic keratosis, basal cell carcinoma or squamous cell carcinoma. The controlled release formulation can likewise be applied to a blood vessel to treat or prevent restenosis, retinopathies or atherosclerosis. The controlled release formulation with appropriated selected neutron capture agent can be used to coat a transplanted organ or tissue to prevent rejection. It can alternatively be implanted or otherwise applied near the site of rheumatoid arthritis.

The field of biodegradable polymers has developed rapidly since the synthesis and biodegradability of polylactic acid was first reported in 1966 by Kulkarni et al. "Polylactic acid for surgical implants," Arch. Surg., *93*, 839. Several other polymers are now known to biodegrade, such as polyanhydrides and polyorthoesters, which take advantage of labile backbone linkages (see: Domb et al. Macromolecules, *22*, 3200, **1989**; and Heller et al. Biodegradable Polymers as Drug Delivery Systems, Dekker, NY: **1990**). Several polymers which degrade into naturally occurring materials have also been described, such as crosslinking gelatin, hyaluronic acid (della Valle et al. U.S. Patent No. 4,987,744 and U.S. Patent No. 4,957,744) and polyaminoacids (Miyake et al., **1974**), which spurred the usage of polyesters by Holland et al. Controlled Release, *4*, 155, **1986** and alph-hydroxy acids (i.e. lactic acid and glycolic acid), which remain the most widely used biodegradable materials for applications ranging from closure devices (sutures and staples) to drug delivery systems (Smith et al. U.S. Patent No. 4,741,337; Spilizeqski et al. J. Control. Rel., *2*, 197, **1985**).

These polymers can be tailored to degrade at a desired rate and with a desired kinetics by selecting the appropriate monomers, method of preparation and molecular weight. Differences in crystallinity of the monomer can alter the polymeric degradation rate. Due to the relatively hydrophobic nature of most polymers, actual mass loss can begin with the oligomeric fragments that are small enough to be water soluble; hence, even the initial molecular weight can influence the degradation rate.

Hydrogels can be used in controlled release formulations. Such polymers are formed from macromers with a polymerizable, non-degradable, region that is separated by at least one degradable region. For example, the water soluble, non-degradable, region

can form the central core of the macromer and have at least two degradable regions which are attached to the core, such that upon degradation, the non-degradable regions (in particular a polymerized gel) are separated. Specifically, as disclosed in U.S. Patent No. 5,626,863 to Hubbell et al., the macromers are PEG-oligoglycolyl-acrylates, with the appropriate end caps to permit rapid polymerization and gelation. Acrylates can be polymerized readily by several initiating systems such as eosin dye, ultraviolet or visible light. The polyethyleneglycol (PEG) is highly hydrophilic and biocompatible. The oligoglycolic acid is a poly(a-hydroxy acid) which can be readily degraded by hydrolysis of the ester linkage into glycolic acid, a nontoxic metabolite. Other chain extensions include polylactic acid, polycaprolactone, polyorthoesters, polyanhydrides and polypeptides. This entire network can be gelled into a biodegradable network that can be used to entrap and homogeneously disperse water-soluble drugs for delivery at a controlled rate. Further, the gel can entrap particulate suspensions of water-insoluble drugs. (See also: U.S. Patent No. 4,591,496 to Cohen *et al.* (Process for Making Systems for the Controlled Release of Macromolecules); U.S. Patent No. 5,545,442 to Van Savage *et al.* (Method for Using a Radiation Cured Drug Release Controlling Membrane); U.S. Patent No. 5,330,768 to Park *et al.* (Controlled Drug Delivery Using Polymer/Pluronic Blends); U.S. Patent No. 5,122,367 to Ron *et al.* (Polyanhydride Bioerodible Controlled Release Implants for Administration of Stabilized Growth Hormone); U.S. Patent No. 5,545,409 to Laurencin *et al.* (Delivery System for Controlled Release of Bioactive Factors); U.S. Patent No. 5,629,009 to Laurencin *et al.* (Delivery System for Controlled Release of Bioactive Factors).

Alternatively, delivery of biologically active substances, both *in vitro* and *in vivo*, via encapsulation has been well described in the prior art. U.S. Patent No. 4,352,883 to Lim et al. entitled "Encapsulation of Biological Material" discloses the encapsulation of proteins within a membrane by suspending the protein in an aqueous medium containing a water-soluble gum that can be reversibly gelled to form the suspension into droplets. These droplets can be gelled further into discrete, shape-retaining, water insoluble temporary capsules with the aid of a solution of multivalent cations. The temporary capsules then can be further wrapped by an ionically cross-linking surface layer to form a semipermeable membrane around the capsules that is permeable to small molecules but impermeable to larger molecules. Microencapsulations of glycoproteins have also been well described. U.S. Patent No. 4,324,683 to Lim et al. entitled "Encapsulation of Labile

Biological Material" encapsulates a glycoprotein by a two-step interfacial polymerization process to form capsules with well-controlled porosity. The microcapsules serve to protect the active substances from attack by microorganisms and from any immunological response. U.S. Patent No. 5,718,921 to Mathiowitz *et al.* (Microspheres Comprising Polymer and Drug Dispersed There Within) discloses a method to encapsulate relatively temperature-labile drugs into a microsphere.

Several methods have been developed to reversibly encapsulate biologically active substances. One that can be applied both to in vitro and in vivo studies has been described in U.S. Patent No. 4,900,556 by Wheatley et al. entitled "System for Delayed and Pulsed Release of Biologically-Active Substances." In this disclosed system, the biologically-active substance can be released either at a constant rate over a period of time or in discrete pulses. The biologically active materials are entrapped within liposomes encapsulated within semipermeable microcapsules or permeable polymeric matrix. Release of the desired materials is governed by the permeability of both the liposome and the surrounding matrix (the matrix integrity is directly proportional to the liposome integrity); the permeability of the liposome can be engineered by modifying the composition and the method for making the liposome to produce liposome that are sensitive to specific stimuli such as temperature, pH or light. For example, by including a phospholipase that degrades the liposome within some or all of the liposomes or the surrounding matrix, the liposome can be destabilized and broken down over a period of time. Other systems have been developed, e.g. U.S. Patent No. 4,933,185 by Wheatley et al., which utilize a core made up of a polymer (such as an ionically cross-linked polysaccharide with calcium alginate or chitin) around which there is an ionically bound skin (such as a polycationic skin of poly-L-lysine) whose integrity is dependent on the core polymer. With an impermeable skin, when the core polymer can be degraded by enzymes (such as alginase from the bacteria, chitinase or hydrolase), there is a sudden release of biologically active substance from the core. Alternatively, the skin can be partially permeable for a gradual release of drug upon degradation of the core.

Nanoparticles are especially useful in the delivery of drugs parenterally or intravenously such that the delivery device is small with a long circulating half-life. A number of injectable drug delivery systems have been investigated, including microcapsules, microparticles, liposomes and emulsions. The major obstacle for these delivery systems is the rapid clearance of the materials from the blood stream by the

macrophages of the reticuloendothelial system (RES). For example, polystyrene particles as small as sixty nanometers in diameter are cleared from the blood within two to three minutes. Liposomal drug delivery systems have also been extensively studied for this application because they were expected to freely circulate in the blood. Coating of the liposomes with poly(ethylene glycol) (PEG) increased the half-life of the carriers due to PEG's hydrophobic chains which reduced its protein absorption and thus its RES uptake. U.S. Patent No. 5,543,158 to Gref *et al.* (Biodegradable Injectable Nanoparticles) describes a carrier system specifically targeted towards carriers suitable for intravenous delivery with a controlled release mechanism with modified polyglycols.

U.S. Patent No. 5,626,862, U.S. Patent No. 5,651,986 and U.S. Patent No. 5,846,565 to Brem *et al.* (Controlled Local Delivery of Chemotherapeutic Agents for Treating Solid Tumors) discloses the use of these carriers for the specific delivery of chemotherapeutic agents to increase bioavailability. Therefore, the devices act as reservoirs that release drugs over an extended period of time while at the same time preserves the bioactivity and bioavailability of the agent. U.S. Patent No. 5,286,763 to Gerhard *et al.* (Bioerodible Polymers for Drug Delivery in Bone) further discloses that bioerodible polymers can be used to deliver chemotherapeutic agents directly into the bone. Cohen *et al.* U.S. Patent No. 5,562,099 (Polymeric Microparticles Containing Agents for Imaging) discusses the usage of these carriers as contrast agents. The polymeric microparticle is filled with contrast agents for enhanced imaging.

Books describing methods of controlled delivery that are appropriate for the delivery of the TC- or IF-binding carriers/neutron capture agents of the present invention include: Robert S. Langer, Donald L. Wise, editors; Medical applications of controlled release (Volumes 1 and 2); Boca Raton, FL: CRC Press, **1984;** and William J. M. Hrushesky, Robert Langer, and Felix Theeuwes, editors; Temporal control of drug delivery (series); New York: New York Academy of Sciences, 1991.

Nonlimiting examples of U.S. Patents that describe controlled release formulations are: U.S. Patent No. 5,356,630 to Laurencin *et al.* (Delivery System for Controlled Release of Bioactive Factors); ; U.S. Patent No. 5,797,898 to Santini, Jr. *et al.* (Microchip Drug Delivery Devices); U.S. Patent No. 5,874,064 to Edwards *et al.* (Aerodynamically Light Particles for Pulmonary Drug Delivery); U.S. Patent No. 5,548,035 to Kim *et al.* (Biodegradable Copolymer as Drug Delivery Matrix Comprising Polyethyleneoxide and

Aliphatic Polyester Blocks); U.S. Patent No. 5,532,287 to Savage *et al.* (Radiation Cured Drug Release Controlling Membrane); U.S. Patent No. 5,284,831 to Kahl *et al.* (Drug Delivery Porphyrin Composition and Methods); U.S. Patent No. 5,741,329 to Agrawal *et al.* (Methods of Controlling the pH in the Vicinity of Biodegradable Implants); U.S. Patent No. 5,820,883 to Tice *et al.* (Methods for Delivering Bioactive Agents into and Through the Mucosally-Associated Lymphoid Tissues and Controlling Their Release);U.S. Patent No. 5,955,068 to Gouin *et al.* (Biodegradable Polyanhydrides Derived from Dimers of Bile Acids, and Use Thereof as Controlled Drug Release Systems); U.S. Patent No. 6,001,395 to Coombes *et al.* (Polymeric Lamellar Substrate Particles for Drug Delivery); U.S. Patent No. 6,013,853 to Athanasiou *et al.* (Continuous Release Polymeric Implant Carriers); U.S. Patent No. 6,060,582 to Hubbell *et al.* (Photopolymerizable Biodegradable Hydrogels as Tissue Contacting Materials and Controlled Release Carriers); U.S. Patent No. 6,113,943 to Okada *et al.* (Sustained-Release Preparation Capable of Releasing a Physiologically Active Substance); and PCT Publication No. WO 99/59548 to Oh *et al.* (Controlled Drug Delivery System Using the Conjugation of Drug to Biodegradable Polyester); U.S. Patent No. 6,123,861 (Fabrication of Microchip Drug Delivery Devices); U.S. Patent No. 6,060,082 (Polymerized Liposomes Targeted to M cells and Useful for Oral or Mucosal Drug Delivery); U.S. Patent No. 6,041,253 (Effect of Electric Field and Ultrasound for Transdermal Drug Delivery); U.S. Patent No. 6,018,678 (Transdermal protein delivery or measurement using low-frequency sonophoresis); U.S. Patent No. 6,007,845 Nanoparticles And Microparticles Of Non-Linear Hydrophilic-Hydrophobic Multiblock Copolymers; U.S. Patent No. 6,004,534 Targeted Polymerized Liposomes For Improved Drug Delivery; U.S. Patent No. 6,002,961 Transdermal Protein Delivery Using Low-Frequency Sonophoresis; U.S. Patent No. 5,985,309 Preparation Of Particles For Inhalation; U.S. Patent No. 5,947,921 Chemical And Physical Enhancers And Ultrasound For Transdermal Drug Delivery; U.S. Patent No. 5,912,017 Multiwall Polymeric Microspheres; U.S. Patent No. 5,911,223 Introduction Of Modifying Agents Into Skin By Electroporation; U.S. Patent No. 5,874,064 Aerodynamically Light Particles For Pulmonary Drug Delivery; U.S. Patent No. 5,855,913 Particles Incorporating Surfactants For Pulmonary Drug Delivery; U.S. Patent No. 5,846,565 Controlled Local Delivery Of Chemotherapeutic Agents For Treating Solid Tumors; U.S. Patent No. 5,837,752 Semi-Interpenetrating Polymer Networks; U.S. Patent No. 5,814,599 Transdermal Delivery Of Encapsulated Drugs; U.S. Patent No. 5,804,178 Implantation Of Cell-Matrix Structure Adjacent Mesentery, Omentum Or Peritoneum Tissue; U.S. Patent No. 5,797,898

Microchip Drug Delivery Devices; U.S. Patent No. 5,770,417 Three-Dimensional Fibrous Scaffold Containing Attached Cells For Producing Vascularized Tissue In Vivo; U.S. Patent No. 5,770,193 Preparation Of Three-Dimensional Fibrous Scaffold For Attaching Cells To Produce Vascularized Tissue In Vivo; U.S. Patent No. 5,762,904 Oral Delivery Of Vaccines Using Polymerized Liposomes; U.S. Patent No. 5,759,830 Three-Dimensional Fibrous Scaffold Containing Attached Cells For Producing Vascularized Tissue In Vivo; U.S. Patent No. 5,749,847 Delivery Of Nucleotides Into Organisms By Electroporation; U.S. Patent No. 5,736,372 Biodegradable Synthetic Polymeric Fibrous Matrix Containing Chondrocyte For In Vivo Production Of A Cartilaginous Structure; U.S. Patent No. 5,718,921 Microspheres Comprising Polymer And Drug Dispersed There Within; U.S. Patent No. 5,696,175 Preparation Of Bonded Fiber Structures For Cell Implantation; U.S. Patent No. 5,667,491 Method For Rapid Temporal Control Of Molecular Transport Across Tissue; U.S. Patent No. 5,654,381 Functionalized Polyester Graft Copolymers; U.S. Patent No. 5,651,986 Controlled Local Delivery Of Chemotherapeutic Agents For Treating Solid Tumors; U.S. Patent No. 5,629,009 Delivery System For Controlled Release Of Bioactive Factors; U.S. Patent No. 5,626,862 Controlled Local Delivery Of Chemotherapeutic Agents For Treating Solid Tumors; U.S. Patent No. 5,593,974 Localized Oligonucleotide Therapy; U.S. Patent No. 5,578,325 Nanoparticles And Microparticles Of Non-Linear Hydrophilic-Hydrophobic Multiblock Copolymers; U.S. Patent No. 5,562,099 Polymeric Microparticles Containing Agents For Imaging; U.S. Patent No. 5,545,409 Delivery System For Controlled Release Of Bioactive Factors; U.S. Patent No. 5,543,158 Biodegradable Injectable Nanoparticles; U.S. Patent No. 5,514,378 Biocompatible Polymer Membranes And Methods Of Preparation Of Three Dimensional Membrane Structures; U.S. Patent No. 5,512,600 Preparation Of Bonded Fiber Structures For Cell Implantation; U.S. Patent No. 5,500,161 Method For Making Hydrophobic Polymeric Microparticles; U.S. Patent No. 5,487,390 Gas-filled polymeric microbubbles for ultrasound imaging; U.S. Patent No. 5,399,665 Biodegradable polymers for cell transplantation; U.S. Patent No. 5,356,630 Delivery system for controlled release of bioactive factors; U.S. Patent No. 5,330,768 Controlled drug delivery using polymer/pluronic blends; U.S. Patent No. 5,286,763 Bioerodible polymers for drug delivery in bone; U.S. Patent No. 5,149,543 Ionically cross-linked polymeric microcapsules; U.S. Patent No. 5,128,420 Method of making hydroxamic acid polymers from primary amide polymers; U.S. Patent No. 5,122,367 Polyanhydride bioerodible controlled release implants for administration of stabilized growth hormone;

U.S. Patent No. 5,100,668 Controlled release systems containing heparin and growth factors; U.S. Patent No. 5,019,379 Unsaturated polyanhydrides; U.S. Patent No. 5,010,167 Poly(amide-and imide-co-anhydride) for biological application; .S. Patent No. 4,948,587 Ultrasound enhancement of transbuccal drug delivery; U.S. Patent No. 4,946,929 Bioerodible articles useful as implants and prostheses having predictable degradation rates; U.S. Patent No. 4,933,431 One step preparation of poly(amide-anhydride); U.S. Patent No. 4,933,185 System for controlled release of biologically active compounds; U.S. Patent No. 4,921,757 System for delayed and pulsed release of biologically active substances; U.S. Patent No. 4,916,204 Pure polyanhydride from dicarboxylic acid and coupling agent; U.S. Patent No. 4,906,474 Bioerodible polyanhydrides for controlled drug delivery; U.S. Patent No. 4,900,556 System for delayed and pulsed release of biologically active substances; U.S. Patent No. 4,898,734 Polymer composite for controlled release or membrane formation; U.S. Patent No. 4,891,225 Bioerodible polyanhydrides for controlled drug delivery; U.S. Patent No. 4,888,176 Controlled drug delivery high molecular weight polyanhydrides; .S. Patent No. 4,886,870 Bioerodible articles useful as implants and prostheses having predictable degradation rates; U.S. Patent No. 4,863,735 Biodegradable polymeric drug delivery system with adjuvant activity; U.S. Patent No. 4,863,611 Extracorporeal reactors containing immobilized species; U.S. Patent No. 4,861,627 Preparation of multiwall polymeric microcapsules; U.S. Patent No. 4,857,311 Polyanhydrides with improved hydrolytic degradation properties; U.S. Patent No. 4,846,786 Bioreactor containing suspended, immobilized species; U.S. Patent No. 4,806,621 Biocompatible, bioerodible, hydrophobic, implantable polyimino carbonate article; U.S. Patent No. 4,789,724 Preparation of anhydride copolymers; U.S. Patent No. 4,780,212 Ultrasound enhancement of membrane permeability; U.S. Patent No. 4,779,806 Ultrasonically modulated polymeric devices for delivering compositions; U.S. Patent No. 4,767,402 Ultrasound enhancement of transdermal drug delivery; U.S. Patent No. 4,757,128 High molecular weight polyanhydride and preparation thereof; .S. Patent No. 4,657,543 Ultrasonically modulated polymeric devices for delivering compositions; U.S. Patent No. 4,638,045 Non-peptide polyamino acid bioerodible polymers; U.S. Patent No. 4,591,496 Process for making systems for the controlled release of macromolecules.

The invention may be further illustrated by the following examples.

EXAMPLES

Ultraviolet-visible spectra were recorded with a diode array spectrophotometer. ^{11}B NMR spectra at 192.656 MHZ with ^{1}H decoupling were recorded on Varian 1NOVA 600 MHZ spectrometer with an 8 mm broad band probe. Approximately 10 mg of the cobalamin-conjugate were dissolved in 1 mL pyridine d_6 in a 5 mm NMR tube. Five hundred scans were collected with an acquisition time of 0.133 seconds and a relaxation delay of 1 second. Chemical shifts are given relative to BF_3:$(CH_3CH_2)_2O$.

Mass spectral data was obtained on a Sciex API 365 LC-MS/MS system (Toronto, Canada). Separations were done on a Shimadzu HPLC system consisting of two LC-10AD pumps and a SCL-10Avp controller (Shimadzu Scientific Instruments, Columbia, MD). Analytes were monitored by UV at 214 NM with an ABI 785 detector. HPLC separations were achieved using a BDS-Hypersil C8 column (150 × 4.6 mm; 120 A, Keystone Scientific, Inc., Bellefonte, PA). The mobile phase consisted of H_2O:methanol (98:2 v:v) in pump A and H_2O: methanol (2:92 v:v) in pump B. A linear gradient was used from 5% B to 30% B over 10 minutes and was held at 30% B for 20 minutes before returning to initial conditions (mono-carborane synthesis). The di-carborane required a longer gradient using the same mobile phases; 5% B to 65% B over 25 minutes and held at 65% B for 15 minutes before returning to initial conditions. The separations were monitored by UV absorption at 214 NM. The flow was 1.0 mL/minute and was split post-column allowing ~ 10 μ to flow into the mass spectrometer.

Mass spectral data was collected using electrospray ionization in positive mode over a mass range of 300 to 2300 AMU at a dwell time of 0.3 ms/0.1 AMU. Synthetic samples were prepared at 5 or 10 mg/mL in pump A mobile phase and an aliquot injected onto the HPLC (1-5 μL). Retention times of the mono- and di-carborane products were determined to be 13.3 minutes and 16.2 minutes respectively. Purification of the b and d mono-carborane products was achieved by collecting fractions at the elution times for several injections. The collected fractions were combined and dried to a powder. A portion of the purified product was dissolved in methanol:H_2O (1:1) and reanalyzed by HPLC-MS to ascertain the purity.

o-Carborane, butyllithium (1.6 M solution in hexanes) and putrescine were purchased from Aldrich Chemical Company (Milwaukee, WI). The water-soluble

carbodiimide 1-ethyl-3(3'-dimethylaminopropyl) carbodiimide and 1-hydroxybenzotriazole were from Sigma (St. Louis, MO). Thin layer chromatography (TLC) silica gel plates were obtained from Eastman Kodak Company. The cyanocobalamin-b, d, and e monocarboxylic acids and the b, d-dicarboxylic acid were prepared as described before (see U.S. Patent Number 5,739,313, and D. L. Anton, et al., *J. Am. Chem. Soc.*, **1980**, *102*, 2212-2219), and were provided by Professor Kahl of the Department of Medicinal Chemistry at the University of California, San Francisco.

Example 1

o-Carborane carboxylic acid (2).

A solution of o-carborane (**1**) (5.0 g, 34.7 mmol) in 500 mL dry ether in a 1 L round bottom flask was cooled to -78°C in a dry ice-acetone bath. The solution was flushed with argon and the flask sealed with a serum stopper. n-Butyllithium (24 mL, 1.6 M in hexanes) was slowly injected via a syringe over a period of about 20 minutes and the reaction was stirred for an additional 30 minutes. Crushed dry ice (10-15 g) was then added and the mixture stirred for 1 hour. The dry ice-acetone bath was removed and the reaction allowed to come to room temperature. The ether and hexanes were removed on a rotary evaporator, water (150 mL) was added and unreacted o-carborane was removed by extraction with hexanes (2 × 100 mL). The aqueous phase was acidified with concentrated HCl and the desired product extracted with hexanes (4 × 100 mL). The combined extracts were dried over Na_2SO_4 and evaporated to dryness to give 5.8 g (88.7%) of o-carborane carboxylic acid (**2**), which was used without further purification.

Example 2

o-Carborane carboxylic acid chloride (3).

o-Carborane carboxylic acid (2.0 g, 10.6 mmol), dried over P_2O_5, was dissolved in 30 mL thionyl chloride and heated under reflux for 3 hours. The solution was cooled to

room temperature, evaporated to dryness and dried over P_2O_5 (2.05 g, 9.9 mmol, 93%) to provide (3), which was used without further purification.

Example 3

Nido-Carboranoyl(4-aminobutyl) amide (4).

The acid chloride (3) was dissolved in 15 mL dry pyridine and 1,4-diaminobutane (1.12 g, 12.7 mmol) was added. The reaction mixture was heated under reflux for 3 hours, cooled to room temperature and concentrated. Water (10 mL) was added and the suspension acidified with 3 M HCl. The desired product was extracted with ethyl acetate (4 × 50 mL), the combined organic layers were washed once with water, dried over Na_2SO_4 and evaporated to dryness to yield 2.35 g (8.0 mmol, 75%) of (4). Thus far the product has resisted crystallization from a variety of solvent mixtures, however TLC on silica gel plates (2-propanol-NH_4OH-H_2O; 7:1:2) showed only one ninhydrin positive compound distinct from the diamine.

Example 4

*Cyanocobalamin-nido-carborane conjugates (5, **Figure 2**).*

Separate reaction mixtures containing 1 g (~0.66 mmol) of the b-monocarboxylic acid, d-monocarboxylic acid, or the b,d-dicarboxylic acid of cyanocobalamin, hydroxybenzotriazole (810 mg, 6.0 mmol), 1-ethyl-3(3-dimethylaminopropyl) carbodiimide (11.4 g, 6.0 mmol) and 4 (600 mg, 2.0 mmol) in 100 mL of a water-acetone mixture (2:1) were adjusted to pH 6.9 with 1N NaOH. The reactions were stirred at room temperature and their progress monitored by TLC. After 3 hours, the mixtures were concentrated to remove acetone and the resulting aqueous suspensions were extracted into 92% aqueous phenol. The phenol phases were washed with water to remove the water-soluble reactants. One volume of acetone and three volumes of ether were added to each of the phenol phases and the desired cyanocobalamin-nido-carborane conjugates were

back extracted into water. The aqueous layers were extracted three times with ether to remove residual phenol and unreacted (4). Finally the aqueous solutions were evaporated to dryness. The residuals were triturated with acetone and the desired conjugates isolated as orange-red powders (yields 90-95% based on the cyanocobalamin-carboxylic acids).

The isotope ratios of the final products were also compared to theoretical as follows: Mono product theoretical $M+H^+$ (C70H109CoB9O15PN15) - 1584.8 (12%), 1585.8 (37%), 1586.8 (74%), 1587.8 (100%), 1588.8 (84%), 1589.8 (44%), 1590.8 (16%), 1591.8 (4%). Synthesis I - 1584.8 (14%), 1585.8 (37%), 1586.8 (74%), 1587.8 (100%), 1588.8 (88%), 1589.8 (48%), 1590.8 (19%), 1591.8 (6%). Synthesis II - 1584.8 (15%), 1585.8 (40%), 1586.8 (78%), 1587.8 (100%), 1588.8 (86%), 1589.8 (44%), 1590.8 (17%), 1591.8 (7%).

Di product theoretical $M+H^+$ - (C77H129CoB18O16PN16) - 1815.1 (9%), 1816.1 (23%), 1817.1 (47%), 1818.1 (77%), 1819.1 (99%), 1820.1 (100%), 1821.1 (77%), 1822.1 (44%), 1823.1 (19%), 1824.1 (6%). Observed synthesis - 1815.1 (15%), 1816.1 (27%), 1817.1 (51%), 1818.1 (79%), 1819.1 (100%), 1820.1 (100%), 1821.1 (78%), 1822.1 (49%), 1823.1 (25%), 1824.1 (12%).

Results included the identification of the *b* and *d* carborane cyanocobalamin (CCC) analogs by LC-MS. Products identified were subsequently separated and purified by HPLC. The purified products were reanalyzed by LC-MS with no starting material detected. MS/MS data was also obtained on the *b* and *d* CCC analogs to provide further structural characterization. These purified products were then tested in the biological assays. The *e*-CCC analog and the *di*-CCC analog were also separated and identified by LC-MS, however, the preparations contained more reaction side products resulting in difficult purification. The isotope ratios of the *b*- and *d*-CCC analogs, as well as the *di*-CCC analog, were compared to theoretical isotope ratios to provide further information for identification.

Mass spectra confirmed the presence of each cyanocobalamin-nido-carborane conjugate. Mass spectroscopy analysis as well as ^{11}B NMR showed that during the conversion of **3** to **4** (**Figures 2** and **3**) the o-carborane nucleus lost a boron atom to yield the nido-carborane derivative **4** (**Figure 3**). UV-visible spectroscopy of the final products

showed maxima typical of cyanocobalamin, indicating that the corrin nucleus was intact and that the 5,6-dimethyl benzimidazole nucleotide was still attached to the cobalt atom.

Example 5

6-(3-Aminoprop-1-yl)cobalamin.

Cyano cobalamin (500 mg) was dissolved in 100 mL deoxygenated water containing 10 mg $CoCl_2$, and reduced with sodium borohydride to give the corresponding Co (I) compound. After 30 minutes, 3-chloropropyl amine hydrochloride (130 mg) dissolved in 5 mL deoxygenated ethanol was added. After one hour, at room temperature, the mixture was desalted via phenol extraction. The aminopropylcarbolamine was back extracted into water after the addition of 1 volume of acetone and 3 volumes of ether. The aqueous solution was concentrated and the desired 6-(3-aminoprop-1-yl)cobalamin crystallized from aqueous acetone (yield 510 mg).

Example 6

6-Carboranoylamidopropyl cobalamin.

6-(3-aminoprop-1-yl)cobalamin (300 mg), hydroxybenzotriazole (270 mg), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (382 mg) and o-carborane monocarboxylic acid were dissolved in water (50 mL) and acetone (20 mL) and allowed to react at room temperature for 3 hours. The reaction mixture was concentrated to remove acetone and desalted via phenol extraction. The concentrated aqueous solution was crystallized from aqueous acetone to give the title compound.

Example 7

DTPA-aminopropylcobalamin (DAPC)

Using a coupling procedure similar to that described in Example 2, 6-(3-aminoprop-1-yl)cobalamin and DTPA were coupled in the presence of hydroxybenzotriazole to give the title compound.

Example 8

In vitro biological activity of the carborane cyanocobalamin analogs.

To assess the *in vitro* binding of the carborane cyanocobalamin (**Example 1**, CCC) and DTPA-aminopropylcobalamin (**Example 3**, DAPC) analogs to the transcobalamin proteins, the unsaturated Vitamin B12 binding capacity (UBBC) assay (see D. A. Collins and H. P. C. Hogenkamp, *J. Nuclear Medicine,* **1997**, *38*, 717-723) was performed. Serum was obtained from 5 patients being evaluated for pernicious anemia at the Mayo Clinic. The patients' serum first underwent a routine clinical UBBC as previously described. To determine if the CCC and DAPC analogs would inhibit Co-57 cyanocobalamin from binding to the transcobalamin proteins, the excess serum from the 5 patients underwent modified UBBC.

Specifically, under dim light, 0.4 mL serum was treated with 4 μL (concentration 10 μg CCC per mL normal saline) of the 2 CCC analogs, carborane-d-cyanocobalamin and carborane-b-cyanocobalamin, and DAPC. The analogs were incubated for 20 minutes at room temperature with the patient's serum. Both the clinical run and the analog treated samples were assayed for UBBC as usual.

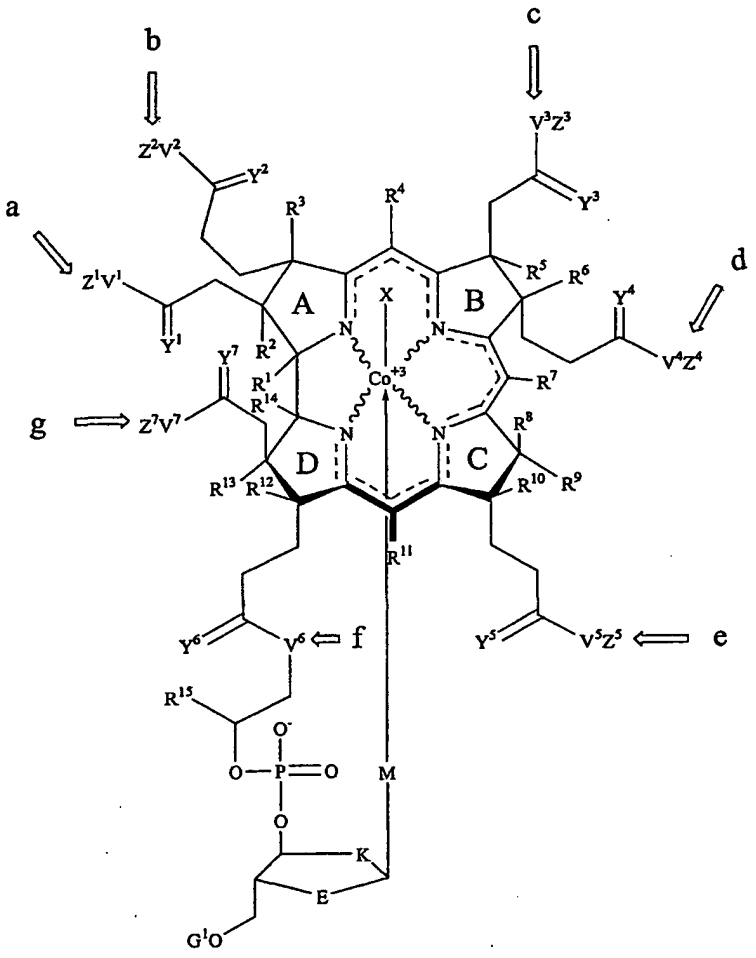
The analogs competitively blocked Co-57-cyanocobalamin from binding to the transcobalamin proteins. Therefore, the cpm of the modified UBBC assay was significantly lower than that of the clinical runs. The percent PB of the analogs to transcobalamin protein was calculated as follows (PB = 100 - CCC_{UBBC} cpm/clinical UBBC cpm × 100). The average percent binding (PB) of the 5 solutions (n = 10 for each solution, i.e., two modified UBBC assays per patient) for the carborane-d cyanocobalamin

and carborane-b cyanocobalamin was 35.75% and 92.93% respectively, and 98.21% for DAPC.

The invention has been described with reference to various specific and preferred embodiments and techniques. However, it should be understood that many variations and modifications may be made while remaining within the spirit and scope of the invention.

WE CLAIM:

1. A compound of the formula (I):



(I)

or its enantiomer, diastereomer or its pharmaceutically acceptable salt, wherein:

(i) the wavy line in the chemical structure indicates either a dative or covalent bond such that there are three dative Co-N bonds and one covalent Co-N bond, wherein, in the case of the dative bond, the valence of nitrogen is completed either with a double bond with an adjacent ring carbon or with a hydrogen;

(ii) the dotted line in the chemical structure indicates either a double or single bond such that the double bond does not over-extend the valence of the element (i.e. to give pentavalent carbons) and, in the case of a single bond, the valence is completed with hydrogen;

(iii) X is hydrogen, cyano, halogen (Cl, F, Br or I), haloalkyl (including CF_3, CF_2CF_3, CH_2CF_3 and CF_2Cl), NO, NO_2, NO_3, phosphonate (including alkyl-$P(O)_2OR^{15}$), $PR^{15}R^{16}R^{17}$, NH_2, $NR^{15}R^{16}$, OH, OR^{15}, SR^{15}, SCN, N_3, $OC(O)R^{15}$, $C(O)_2R^{15}$, $C(O)R^{15}$, $OC(O)NR^{15}R^{16}$, $C(O)_2NR^{15}R^{16}$, $C(O)NR^{15}R^{16}$, $P(O)_2OR^{15}$, $S(O)_2OR^{15}$, a purine or pyrimidine nucleoside or nucleoside analog, adenosyl, 5-FU, alkyl, alkenyl, alkynyl, aryl, aralkyl, alkaryl, amino acid, peptide, protein, carbohydrate, heteroalkyl, heterocycle, heteroaryl or alkylheteroaryl;

(iv) M is a monovalent heterocycle or heteroaromatic, which is capable of binding to the adjacent sugar ring, and forming a dative bond with Co^{+3};

(v) K is O, S, NJ^1, C(OH)H, $CR^{100}R^{101}$ or $C(R^{100})V^8Z^8$;

(vi) E is O or S;

(vii) G^1 is hydrogen, alkyl, acyl, silyl, phosphate or L-T;

(viii) Y^1, Y^2, Y^3, Y^4, Y^5, Y^6 and Y^7 independently are O, S or NJ^2;

(ix) V^1, V^2, V^3, V^4, V^5, V^6, V^7 and V^8 independently are O, S, NJ^3, $CR^{102}R^{103}$ or a direct bond;

(x) Z^1, Z^2, Z^3, Z^4, Z^5, Z^7 and Z^8 independently are R^{104} or L-T;

(xi) each L is independently a direct bond or linker to one or more T moieties, and that does not significantly impair the ability of the TC- or IF-binding carrier to bind to a transcobalamin receptor, optionally when bound to a transport protein;

(xii) each T independently comprises the residue of one or more molecules neutron capture agents;

(xiii) at least one of Z^1, Z^2, Z^3, Z^4, Z^5, Z^7, Z^8, K and G^1 is L-T;

(xiv) J^1, J^2 and J^3 independently are hydrogen, alkyl, alkenyl, alkynyl, alkaryl, cycloalkyl, aryl, cycloaryl, heteroalkyl, heterocycle, heteroaryl, hydroxyl, alkoxy or amine;

(xv) R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{12}, R^{13} and R^{14} independently are hydrogen, lower alkyl, lower alkenyl, lower alkynyl, lower cycloalkyl, heteroalkyl, heterocyclic, lower alkoxy, azido, amino, lower alkylamino, halogen, thiol, SO_2, SO_3, carboxylic acid, C_{1-6} carboxyl, hydroxyl, nitro, cyano, oxime or hydrazine;

(xvi) R^{13} and R^{14} optionally can form a double bond;

(xvii) R^{15}, R^{16} and R^{17} are independently hydrogen, alkyl, alkenyl, alkynyl, aryl, alkaryl or aralkyl group, heteroalkyl, heterocycle or heteroaromatic; and

(xviii) R^{100}, R^{101}, R^{102}, R^{103}, and R^{104} are independently hydrogen, alkyl, alkenyl, alkynyl, aryl, acyl, heteroaromatic, heteroaryl, heteroalkyl, hydroxyl, alkoxy, cyano, azido, halogen, nitro, SO_2, SO_3, thioalkyl or amino;

(xix) wherein at least one of each E, G, K, M, R, V and Y is independently not as it is found in natural vitamin B_{12}.

2. The compound of claim 1, wherein at least one T is a molecule that contains B-10.

3. The compound of claim 2, wherein the molecule that contains B-10 is o-carborane, m-carborane or p-carborane.

4. The compound of claim 1, wherein at least one T is a molecule that contains Gd-157.

5. The compound of any one of claims 1-4, wherein at least one L is independently an amine, a polyamine, an amino acid, a poly(amino acid) or peptide linker;

6. The compound of any one of claims 1-4, wherein at least one –L-T is independently a poly(amino acid) residue bound to one or more T.

7. The compound of claim 6, wherein at least one –L-T is independently a poly-L-lysine -NR'(CH((CH_2)$_4$-NHR')CONR')$_m$R', wherein each R' is independently hydrogen, lower alkyl or T; and m is 2-20.

8. The compound of any one of claims 1-4, wherein at least one –L-T is independently a polyamine residue of the formula -NR'(alkylene-NR')$_n$alkyleneNR'R', wherein each R' is independently hydrogen, lower alkyl or T and n is 1-20.

9. The compound of claim 8, wherein -NR'(alkylene-NR')$_n$alkyleneNR' is selected from the group consisting of -NR'(CH_2)$_3$NR'(CH_2)$_4$NR'(CH_2)$_3$NR'R' (spermine); -NR'(CH_2)$_3$NR'(CH_2)$_4$NR'R' (spermidine); decamethylene tetraamine and pentamethylene hexamine.

10. The compound of any one of claims 1-4, wherein at least one –L-T is independently a diamine residue of the formula -NR'(alkylene)$_x$NR'R', wherein each R' is independently hydrogen, lower alkyl or T and x is 2-20.

11. The compound of claim 10, wherein -NR'(alkylene)$_x$NR'R' is selected from the group consisting of 1,6-diaminohexane, 1,5-diaminopentane, 1,4-diaminobutane and 1,3-diaminopropane.

12. A pharmaceutical composition for the treatment, prophylaxis and/or diagnosis of a proliferative disorder in a host comprising a compound of any one of claims 1-11, or the pharmaceutically acceptable salt thereof, in combination with a pharmaceutically acceptable carrier.

13. A pharmaceutical composition for the treatment, prophylaxis and/or diagnosis of a proliferative disorder in a host comprising a compound of any one of claims 1-11, or the pharmaceutically acceptable salt thereof, optionally in a pharmaceutically acceptable carrier, in combination with one or more other therapeutic and/or diagnostic agent(s).

14. The pharmaceutical composition of claim 12 or 13, wherein the host is a human.

15. A method for the treatment, prophylaxis and/or diagnosis of a proliferative disorder in a host comprising administering an effective amount of a compound of any one of claims 1-11, or the pharmaceutically acceptable salt thereof, in combination with a pharmaceutically acceptable carrier.

16. A method for the treatment, prophylaxis and/or diagnosis of a proliferative disorder in a host comprising administering an effective amount of a compound of any one of claims 1-11, or the pharmaceutically acceptable salt thereof, optionally in a pharmaceutically acceptable carrier, in combination or alternation with one or more other therapeutic and/or diagnostic agent(s).

17. The method of claim 15 or 16, wherein the host is a human.

18. Use of an effective amount of a compound of any one of claims 1-11, or the pharmaceutically acceptable salt thereof, optionally in a pharmaceutically acceptable carrier for the treatment, prophylaxis and/or diagnosis of a proliferative disorder in a host.

19. Use of an effective amount of a compound of any one of claims 1-11, or the pharmaceutically acceptable salt thereof, optionally in a pharmaceutically

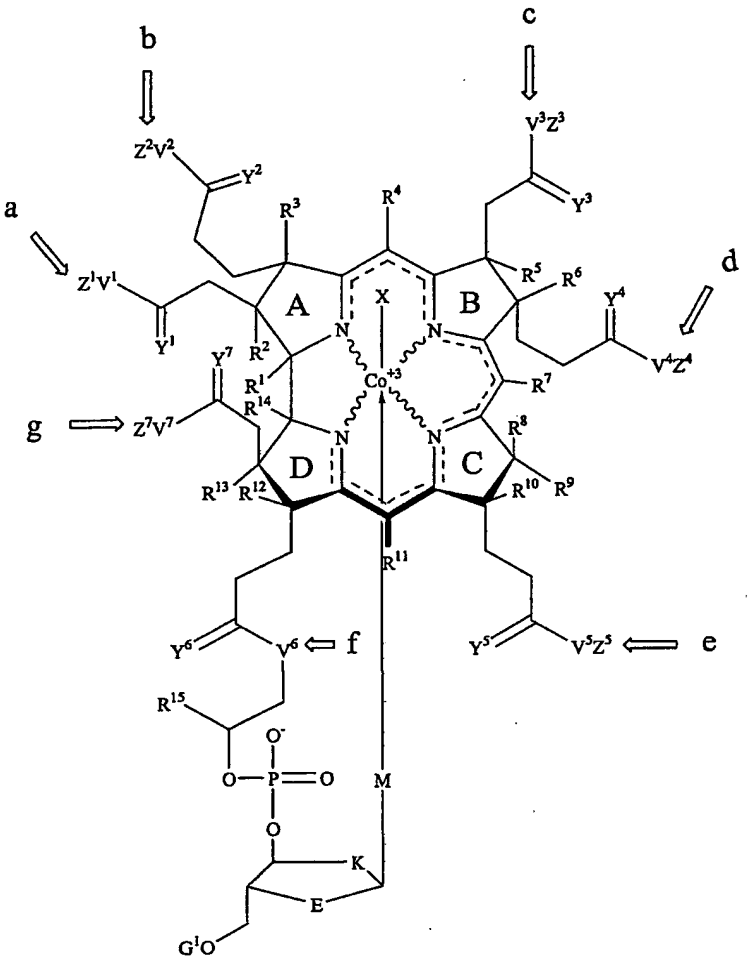
acceptable carrier, in combination or alternation with an effective treatment amount of one or more other therapeutic and/or diagnostic agent(s) for the treatment, prophylaxis and/or diagnosis of a proliferative disorder in a host.

20. Use of an effective amount of a compound of any one of claims 1-11, or the pharmaceutically acceptable salt thereof, optionally in a pharmaceutically acceptable carrier in the manufacture of a medicament for the treatment, prophylaxis and/or diagnosis of a proliferative disorder in a host.

21. Use of an effective amount of a compound of any one of claims 1-11, or the pharmaceutically acceptable salt thereof, optionally in a pharmaceutically acceptable carrier, in combination or alternation with an effective treatment amount of one or more other therapeutic and/or diagnostic agent(s) in the manufacture of a medicament for the treatment, prophylaxis and/or diagnosis of a proliferative disorder in a host.

22. The use of any one of claims 18-21, wherein the host is a human.

23. A pharmaceutical composition for the treatment, prophylaxis and/or diagnosis of a proliferative disorder other than neoplasms in a host comprising a compound of the formula (I):



(I)

or its enantiomer, diastereomer or its pharmaceutically acceptable salt, wherein:

(i) the wavy line in the chemical structure indicates either a dative or covalent bond such that there are three dative Co-N bonds and one covalent Co-N bond, wherein, in the case of the dative bond, the valence of nitrogen is completed either with a double bond with an adjacent ring carbon or with a hydrogen;

(ii) the dotted line in the chemical structure indicates either a double or single bond such that the double bond does not over-extend the valence of the

element (i.e. to give pentavalent carbons) and, in the case of a single bond, the valence is completed with hydrogen;

(iii) X is hydrogen, cyano, halogen (Cl, F, Br or I), haloalkyl (including CF_3, CF_2CF_3, CH_2CF_3 and CF_2Cl), NO, NO_2, NO_3, phosphonate (including alkyl-$P(O)_2OR^{15}$), $PR^{15}R^{16}R^{17}$, NH_2, $NR^{15}R^{16}$, OH, OR^{15}, SR^{15}, SCN, N_3, $OC(O)R^{15}$, $C(O)_2R^{15}$, $C(O)R^{15}$, $OC(O)NR^{15}R^{16}$, $C(O)_2NR^{15}R^{16}$, $C(O)NR^{15}R^{16}$, $P(O)_2OR^{15}$, $S(O)_2OR^{15}$, a purine or pyrimidine nucleoside or nucleoside analog, adenosyl, 5-FU, alkyl, alkenyl, alkynyl, aryl, aralkyl, alkaryl, amino acid, peptide, protein, carbohydrate, heteroalkyl, heterocycle, heteroaryl or alkylheteroaryl;

(iv) M is a monovalent heterocycle or heteroaromatic, which is capable of binding to the adjacent sugar ring, and forming a dative bond with Co^{+3};

(v) K is O, S, NJ^1, C(OH)H, $CR^{100}R^{101}$ or $C(R^{100})V^8Z^8$;

(vi) E is O or S;

(vii) G^1 is hydrogen, alkyl, acyl, silyl, phosphate or L-T;

(viii) Y^1, Y^2, Y^3, Y^4, Y^5, Y^6 and Y^7 independently are O, S or NJ^2;

(ix) V^1, V^2, V^3, V^4, V^5, V^6, V^7 and V^8 independently are O, S, NJ^3, $CR^{102}R^{103}$ or a direct bond;

(x) Z^1, Z^2, Z^3, Z^4, Z^5, Z^7 and Z^8 independently are R^{104} or L-T;

(xi) each L is independently a direct bond or linker to one or more T moieties, and that does not significantly impair the ability of the TC- or IF-binding carrier to bind to a transcobalamin receptor, optionally when bound to a transport protein;

(xii) each T independently comprises the residue of one or more molecules neutron capture agents;

(xiii) at least one of Z^1, Z^2, Z^3, Z^4, Z^5, Z^7, Z^8, K and G^1 is L-T;

(xiv) J^1, J^2 and J^3 independently are hydrogen, alkyl, alkenyl, alkynyl, alkaryl, cycloalkyl, aryl, cycloaryl, heteroalkyl, heterocycle, heteroaryl, hydroxyl, alkoxy or amine;

(xv) R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{12}, R^{13} and R^{14} independently are hydrogen, lower alkyl, lower alkenyl, lower alkynyl, lower cycloalkyl, heteroalkyl, heterocyclic, lower alkoxy, azido, amino, lower alkylamino, halogen, thiol, SO_2, SO_3, carboxylic acid, C_{1-6} carboxyl, hydroxyl, nitro, cyano, oxime or hydrazine;

(xvi) R^{13} and R^{14} optionally can form a double bond;

(xvii) R^{15}, R^{16} and R^{17} are independently hydrogen, alkyl, alkenyl, alkynyl, aryl, alkaryl or aralkyl group, heteroalkyl, heterocycle or heteroaromatic; and

(xviii) R^{100}, R^{101}, R^{102}, R^{103}, and R^{104} are independently hydrogen, alkyl, alkenyl, alkynyl, aryl, acyl, heteroaromatic, heteroaryl, heteroalkyl, hydroxyl, alkoxy, cyano, azido, halogen, nitro, SO_2, SO_3, thioalkyl or amino.

24. A pharmaceutical composition for the treatment, prophylaxis and/or diagnosis of a proliferative disorder other than neoplasms in a host comprising a compound of claim 23, or the pharmaceutically acceptable salt thereof, optionally in a pharmaceutically acceptable carrier, in combination with one or more other therapeutic and/or diagnostic agent(s).

25. The pharmaceutical composition of claim 23 or 24, wherein at least one T is a molecule that contains B-10.

26. The pharmaceutical composition of claim 25, wherein the molecule that contains B-10 is o-carborane, m-carborane or p-carborane.

27. The pharmaceutical composition of claim 23 or 24, wherein at least one T is a molecule that contains Gd-157.

28. The pharmaceutical composition of any one of claims 23-27, wherein at least one L is independently an amine, a polyamine, an amino acid, a poly(amino acid) or peptide linker;

29. The pharmaceutical composition of any one of claims 23-27, wherein at least one -L-T is independently a poly(amino acid) residue bound to one or more T.

30. The pharmaceutical composition of claim 29, wherein at least one –L-T is independently a poly-L-lysine -NR'(CH((CH$_2$)$_4$-NHR')CONR')$_m$R', wherein each R' is independently hydrogen, lower alkyl or T; and m is 2-20.

31. The pharmaceutical composition of any one of claims 23-27, wherein at least one -L-T is independently a polyamine residue of the formula -NR'(alkylene-NR')$_n$alkyleneNR'R', wherein each R' is independently hydrogen, lower alkyl or T and n is 1-20.

32. The pharmaceutical composition of claim 31, wherein -NR'(alkylene-NR')$_n$-alkyleneNR' is selected from the group consisting of -NR'(CH$_2$)$_3$NR'(CH$_2$)$_4$-NR'(CH$_2$)$_3$NR'R' (spermine); -NR'(CH$_2$)$_3$NR'(CH$_2$)$_4$NR'R' (spermidine); decamethylene tetraamine and pentamethylene hexamine.

33. The pharmaceutical composition of any one of claims 23-27, wherein at least one -L-T is independently a diamine residue of the formula -NR'(alkylene)$_x$NR'R', wherein each R' is independently hydrogen, lower alkyl or T and x is 2-20.

34. The pharmaceutical composition of claim 33, wherein -NR'(alkylene)$_x$NR'R' is selected from the group consisting of 1,6-diaminohexane, 1,5-diaminopentane, 1,4-diaminobutane and 1,3-diaminopropane.

35. The pharmaceutical composition of any one of claims 23-34, wherein the host is a human.

36. A method for the treatment, prophylaxis and/or diagnosis of a proliferative disorder in a host comprising administering an effective amount of a compound of any one of claims 23-34, or the pharmaceutically acceptable salt thereof, in combination with a pharmaceutically acceptable carrier.

37. A method for the treatment, prophylaxis and/or diagnosis of a proliferative disorder in a host comprising administering an effective amount of a compound of any one of claims 23-34, or the pharmaceutically acceptable salt thereof, optionally in a pharmaceutically acceptable carrier, in combination or alternation with one or more other therapeutic and/or diagnostic agent(s).

38. The method of claim 36 or 37, wherein the host is a human.

39. Use of an effective amount of a compound of any one of claims 23-34, or the pharmaceutically acceptable salt thereof, optionally in a pharmaceutically acceptable carrier for the treatment, prophylaxis and/or diagnosis of a proliferative disorder in a host.

40. Use of an effective amount of a compound of any one of claims 23-34, or the pharmaceutically acceptable salt thereof, optionally in a pharmaceutically acceptable carrier, in combination or alternation with an effective treatment amount of one or more other therapeutic and/or diagnostic agent(s) for the treatment, prophylaxis and/or diagnosis of a proliferative disorder in a host.

41. Use of an effective amount of a compound of any one of claims 23-34, or the pharmaceutically acceptable salt thereof, optionally in a pharmaceutically

acceptable carrier in the manufacture of a medicament for the treatment, prophylaxis and/or diagnosis of a proliferative disorder in a host.

42. Use of an effective amount of a compound of any one of claims 23-34, or the pharmaceutically acceptable salt thereof, optionally in a pharmaceutically acceptable carrier, in combination or alternation with an effective treatment amount of one or more other therapeutic and/or diagnostic agent(s) in the manufacture of a medicament for the treatment, prophylaxis and/or diagnosis of a proliferative disorder in a host.

43. The use of any one of claims 39-42, wherein the host is a human.

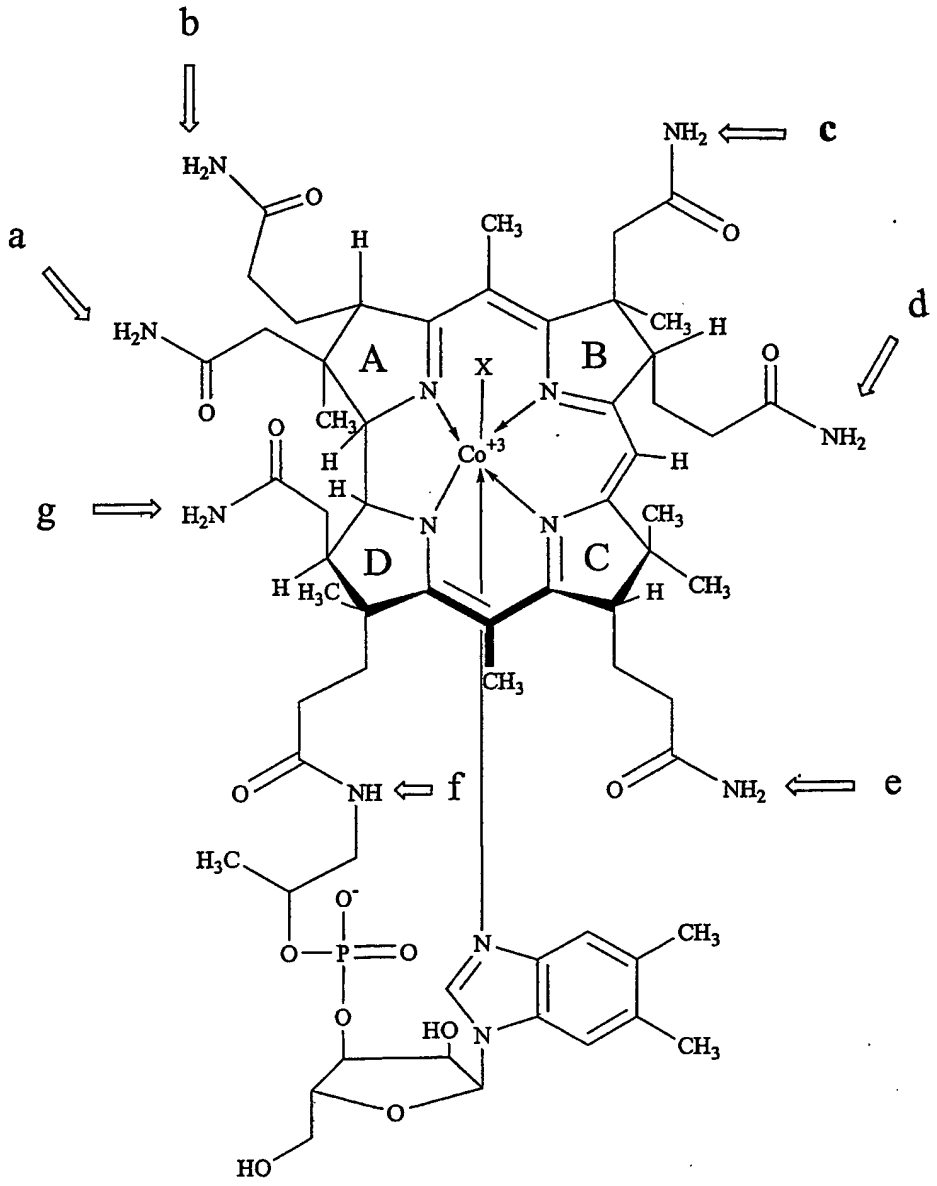


FIGURE 1

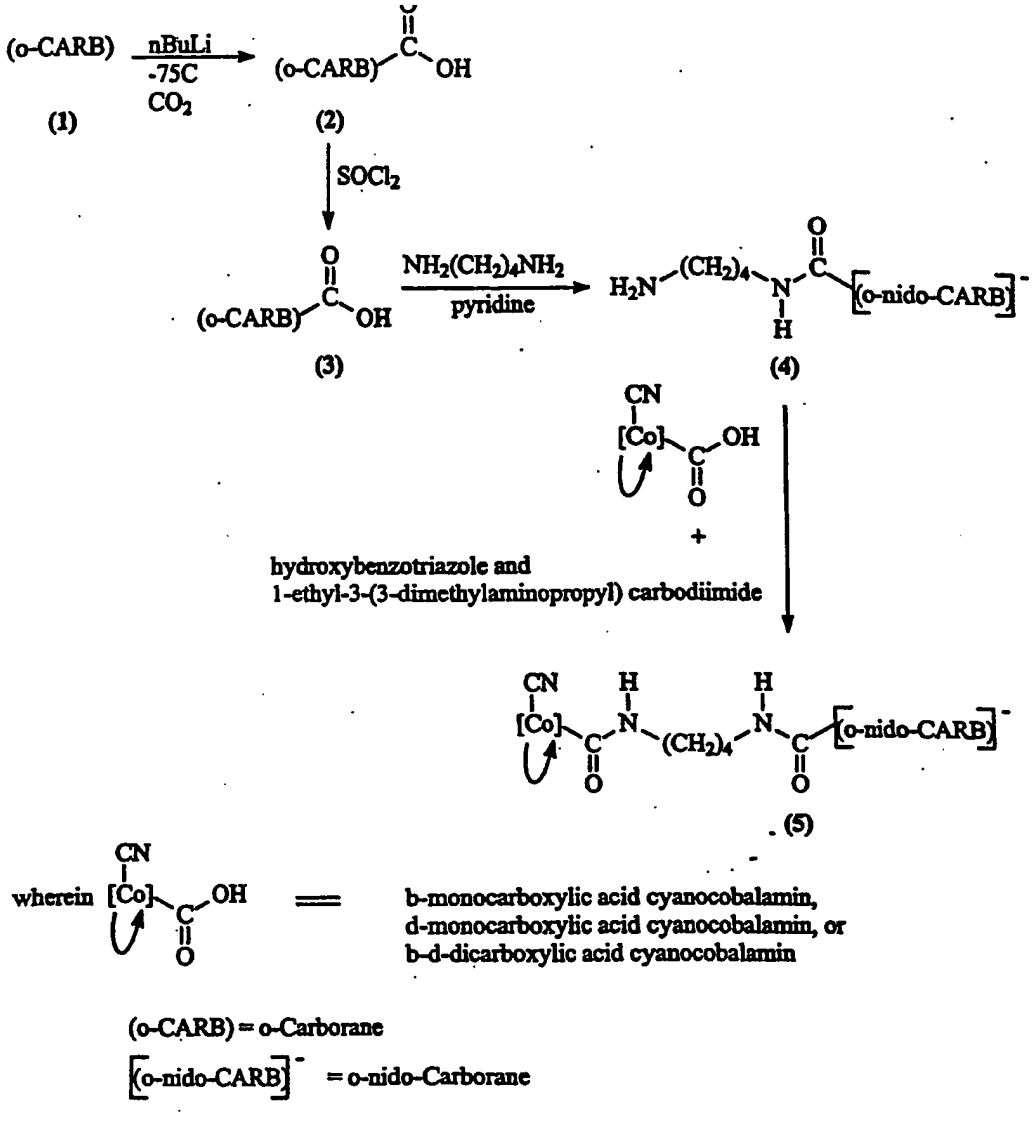

(o-CARB)
nBuLi
-75C
CO2
(1)
(2)
SOCl2
(3)
NH2(CH2)4NH2
pyridine
(4)
hydroxybenzotriazole and
1-ethyl-3-(3-dimethylaminopropyl) carbodiimide
(5)
wherein
b-monocarboxylic acid cyanocobalamin,
d-monocarboxylic acid cyanocobalamin, or
b-d-dicarboxylic acid cyanocobalamin
(o-CARB) = o-Carborane
[o-nido-CARB]- = o-nido-Carborane

FIG. 2

Figure 3